Special issue devoted to the Fourth International Congress of Mediterranean Citriculture, Israel, May 20—27, 1956

• Volume 5D, Number 2—3, March—June 1956

BULLETIN OF THE RESEARCH COUNCIL OF ISRAEL

Section D BOTANY

Bull. Res. Counc. of Israel. D. Bot.

The Weizmann Science Press of Israel · Jerusalem

BULLETIN OF THE RESEARCH COUNCIL OF ISRAEL

יוצא לאור ע"י
מוסד ויצמן לפרסומים במדעי הטבע והטכניקה בישראל
המועצה המדעית לישראל • משרד החנוך והתרבות • האוניברסיטה העברית בירושלים
הטכניון—מכון טכנולוגי לישראל • מכון ויצמן למדע • מוסד ביאליק

Published by
THE WEIZMANN SCIENCE PRESS OF ISRAEL
Research Council of Israel • Ministry of Education and Culture
The Hebrew University of Jerusalem • Technion—Israel Institute of Technology
The Weizmann Institute of Science • Bialik Institute

Manuscripts should be addressed:
Executive Editor, The Weizmann Science Press of Israel, P.O.B. 801, Jerusalem
33, King George Ave., Jerusalem (Telephone 62844)

ד. מ. י"ם 02/2559 — 5.56/1500

• Volume 5D, Number 2—3, March—June 1956.

BULLETIN OF THE RESEARCH COUNCIL OF ISRAEL

Section D
BOTANY

Bull. Res. Counc. of Israel. D. Bot.

The Weizmann Science Press of Israel · Jerusalem מוסד ויצמן לפרסומים במדעי הטבע והטכניקה ישראל · ירושלים

CONTENTS

This special issue has been edited by H. R. Oppenheimer, I. Reichert and K. Mendel. It contains mainly contributions on basic problems in citriculture investigated by students of the Hebrew University.

THE ANATOMY AND HISTOLOGY OF HEALTHY AND XYLOPOROSIS AFFECTED PALESTINE SWEET LIME ROOTSTOCKS BUDDED TO SHAMOUTI SWEET ORANGE*

ERNEST WINOCOUR

Division of Plant Pathology, Agricultural Research Station, Rehovot

ABSTRACT

The xyloporosis disease in Palestine sweet lime rootstocks is characterized by thornlike projections or pegs on the cambial face of the bark which fit into corresponding pits on the cambial face of the wood. Anatomically, this peg and pit deformation consists of a triangular mass of phloem tissue that is wedged into the wood, and is accompanied by pathological changes in both wood and bark. Numerous large lesions, that occur in the older wood rings, are located in radial, tangential, and vertical alignment inside the woody cylinder of the rootstock. These lesions are composed of wound-parenchyma-like cells, and are bordered by areas of distorted xylem tissue characterized by abnormally orientated conducting vessels and wood fibres. Similarly distorted xylem tissue is to be found adjacent to the wedged-in phloem peg at the cambial face. Gum impregnation of the altered wood tissues occurs, but rarely involves the lumina of conducting vessels. The phloem peg consists mainly of crushed, necrotic, sieve tubes and companion cells, lying between hyperplastic and hypertrophied phloem rays. Sieve tube necrosis is also found in the functioning phloem tissues adjacent to the peg deformation, and in parts of the functioning phloem zone not associated with peg deformations. Gum impregnation of the affected bark is evident but does not extend across the bud-union into the tissues of the scion. The relationship between the anatomical abnormalities of the affected rootstock and the foliage decline of the tree top is discussed. It is suggested that the alterations in both the wood and bark might well be responsible for the decline of the tree. An outer bark abnormality, peculiar to young diseased rootstocks, is described, and is considered to be of possible diagnostic value in the nursery. The healthy anatomy of Palestine sweet lime rootstocks is also considered in some detail in order to provide a background for the study of the diseased tissues.

INTRODUCTION

The xyloporosis disease of the Shamouti sweet orange (*C. sinensis*) budded to the Palestine sweet lime (*C. limetta*) rootstock was first recognized in Israel in the year 1928, and by 1930 the damage caused by the disease had already reached serious economic proportions (Reichert and Perlberger 1934). Abroad, the disease has been identified in Cyprus and Syria (Reichert and Perlberger 1934), in Brazil and Argentine (Fawcett 1937), and more recently in Florida (DuCharme and Suit 1951). It is also suspected to be present in California (Batchelor and Webber 1948).

The external foliage symptoms are of a nature common to various citrus decline diseases; smallness and yellowing of the leaves, partial leaf defoliation, and die-back

* Abridged version of a M. Sc. thesis presented to the Hebrew University, Faculty of Agriculture, on 1.6.1955.

Received April 4, 1956.

Bull. Res. Counc. of Israel, Vol. 5D, 1956.

of the tops. A detailed description of these symptoms has been presented elsewhere (Reichert 1953). The disease is also characterized by a pitting abnormality in the wood; on the affected Shamouti orange budded to Palestine sweet lime rootstock trees, thorn-like projections appear on the inner face of the rootstock bark which fit into correspond-ing conoid shaped pits on the cambial face of the wood. Xyloporosis in the Shamouti orange budded to Palestine sweet lime rootstock trees has been more or less controlled in Israel by inarching the highly susceptible sweet lime rootstock with the more resistant sour orange stock.

The causal agent of xyloporosis has not been conclusively determined. Although evidence strongly points to the virus nature of the disease (Reichert 1953), its graft-transmissibility still requires fuller experimental proof. More recent observations on the distribution of the disease in Israel, on the varietal susceptibility of various rootstocks and scions, and on the relationship of xyloporosis to other citrus virus diseases causing wood pitting and foliage decline, have been presented by Reichert (1953, et al. 1953, 1954) and others (McClean 1950, Wallace 1951, Childs 1952).

The phenomenon of wood pitting in citrus trees has received attention in recent years, since it is also characteristic of the numerous citrus decline diseases caused by the tristeza (quick decline) virus complex (Costa et al. 1950, Wallace 1951). Although the morphology of various pitting forms associated with disease in citrus has been described and summarized by DuCharme and Knorr (1954), little work has been done on the histopathology of these abnormalities. In view of this, and considering the fact that the pitting phenomenon appears to be a widespread reaction of citrus tissues to virus attack, this anatomical study of one type of pitting — the conoid pitting character-istic of xyloporosis affected Shamouti on Palestine sweet lime rootstock trees — has been undertaken. In order to provide the necessary background for a study of the diseased tissues, the healthy anatomy of Palestine sweet lime rootstocks will also be considered in some detail.

REVIEW OF LITERATURE

Recent literature on healthy citrus anatomy has been closely associated with specific horticultural and pathological problems. Thus, the anatomy of Valencia sweet orange wood and bark has been described by Webber and Fawcett (1935) in connection with their histopathological study of Valencia tissues affected with the psorosis virus; Mendel (1936), in his study of bud-union anatomy in citrus, has presented some observations on the differential anatomy of sour orange and Palestine sweet lime rootstocks; Schneider (1952, 1954) has made a detailed study of the phloem of the sweet orange and sour orange tree trunk in connection with anatomical changes induced by the tristeza virus; Goldschmidt-Blumental (1954), in her study of the bud-union anatomy of Shamouti sweet orange on various rootstocks, has noted structural differences in the wood of different citrus species. Earlier literature on citrus anatomy has been reviewed by Webber and Fawcett (1935).

Reichert and Perlberger (1934) have described three stages in the development of the bark and wood abnormalities characteristic of xyloporosis affected Palestine sweet lime rootstocks. In the first stage, small ovoid depressions occur on the external face of the bark, and, on removing bark from wood, small pegs or protuberances are evident on the inner (cambial) bark face which correspond to small conoid pits on the cambial face of the wood. These abnormalities are massed just underneath the bud-union. In the second stage of the disease, the small depressions on the outer bark face coalesce into larger patches (particularly underneath the bud-union); an abnormal swelling of the bud-union is often observed, and the peg and pit deformations become discoloured dark brown. Profuse pitting in the wood weakens the rootstock trunk and the tree is likely to bend over under the burden of its fruit. In the final stage of the disease, the bark decays, splits, and shows a tendency to peel off; the wood underneath such places dries up and is darkly discoloured. In a later publication, Reichert et al. (1953) have described two other wood pitting forms associated with xyloporosis: (1) shallow, longitudinal grooves or striations in the wood which correspond to similarly shaped projections on the inner bark face, and (2) small projections on the wood which fit into small pin-holes on the inner bark face. This latter pitting form has been called 'inverse xyloporosis' (Reichert et al. 1953). Du Charme (1952), in a report on xyloporosis in Florida, has noted that the peg and pit abnormalities are most numerous just below the bud-union and become progressively less frequent below the soil line. He also reports that as the disease progresses, gum accumulates in the bark of the rootstock, resulting in a brown discoloration of the phloem layer. DuCharme concludes that: "The presence of pits and pegs and the brown gum layer in the bark are important diagnostic symptoms of xyloporosis."

Reichert and Perlberger (1934), in their anatomical study of the diseased tissues of xyloporosis affected trees, have observed, in cross sections, that: "At many places in the wood, lesions 2 to 3 mm in size, filled with brown gum, may be seen". They have also noted that the seasonal growth rings are not rounded, as is usual in normal citrus wood, but are kinked and bent into pointed triangular abnormalities whose brownish apices end in fair sized lesions. According to these authors, a cross section through the peg and pit deformation at the cambium shows that the inner bark or phloem is wedged into the wood; the xylem cells on both sides of the phloem projection are infiltrated with gum and small necrotic lesions are present in the phloem. Reichert and Perlberger (1934) have also offered an explanation as to the mode of formation of the peg and pit abnormality. A more recent anatomical examination of a peg and pit deformation in citrus has been made by Hughes and Lister (1953) on limes affected with die-back disease in the Gold Coast — a citrus virus disease related to the tristeza virus complex. They have reported that the pits in the wood are caused by gumming in the xylem and, to a lesser extent, in the phloem; the gumming apparently starts at the cambium where gum pockets are formed. It is pertinent to note that the psorosis virus also causes the formation of gum deposits, which more or less correspond to seasonal growth rings, in citrus wood (Fawcett and Bitancourt 1943).

METHODS AND MATERIALS

Samples of the wood and bark, of healthy and xyloporosis affected Shamouti tree budded to Palestine sweet lime rootstock, were collected during summer 1954 and winter 1955. The bark and wood samples were taken at 1 cm below the bud-union by driving a rectangular chisel into the rootstock trunk. In addition, a number of bark samples were taken across the bud-union of the tree in order that longitudinal sections of the rootstock and scion tissues together might be obtained. Two types of diseased trees were sampled: (*a*) 17 year old trees, at the Miqve Israel orange grove which were in an advanced state of foliage decline and which showed severe pitting in the wood of the rootstock, and (*b*) 2 year old budlings, at the Qubeiba nurseries which, though vigorously growing, showed typical xyloporosis pitting on the cambial face of the rootstock wood. None of the diseased trees had been previously inarched. A group of Shamouti on Palestine sweet lime rootstock trees, at the Miqve Israel grove, which showed no foliage decline or pitting in the rootstock wood, were sampled as 'healthy' trees. Bark samples alone were fixed in Randolph's Modified Navashin's Solution (Johansen 1940) for 48 hours, washed in running water, and then stored in 70% ethyl alcohol; wood samples were fixed in Rawlins' Solution Number 2 (Rawlins 1933) for 48 hours, and then transferred to a 1 : 1 mixture of glycerine and 50% ethyl alcohol for softening. After about 9 months in the glycerine-alcohol mixture, the wood samples were sufficiently soft for sectioning. Bark samples were sectioned without any previous softening treatment.

Radial and cross sections, 15 to 25 μ thick, were cut by means of a Reichert OmE sliding microtome, and then stained progressively with dilute Heidenhain's haematoxylin and lacmoid (resorcin blue) according to the method of Schneider (1952). Due to the shortage of good grade clove oil in Israel, sections were cleared in carbol-xylol (1 volume of melted phenol crystals to 3 volumes of xylol). This clearing agent, recommended by Sass (1940), was found to be highly satisfactory. The presence of wound gum was determined by the application of a saturated solution of phloroglucin in 18% HCl (Rawlins 1933) to fresh sections of the material.

HEALTHY ANATOMY AND HISTOLOGY

The wood

The main tissue elements comprising the wood are: conducting vessels, wood parenchyma, libriform wood fibres, medullary rays, and crystal idioblasts (Plate I, Figures 3—6).

Conducting vessels. In cross sections, the vessels appear to be either solitary, in radial multiples of 1 to 6, or occasionally in irregular clusters (Plate I, Figure 4). The vessels are uniformly distributed throughout the growth ring, and, as opposed to the observations of Goldschmidt-Blumental (1954), no differences in vessel distribution in the early and late wood could be observed. Solitary vessels are usually hexagonal or circular to radially elongated in shape, but when located in radial groups are invariably elliptical. In the sections examined, their radial diameter varied from 12 to 120 μ. Walls are thick and heavily lignified. In radial section, the vessel members appear to be

of varying length. End plates are generally horizontal. Vessel member walls are copiously pitted but spiral thickenings were not observed.

Wood parenchyma. Wood parenchyma is abundant, frequently contains stored food, and is of three general types: paratracheal, metatracheal, and terminal (with regard to the growth ring) (Plate I, Figures 3—6). Paratracheal parenchyma cells are relatively large, radially to tangentially elongated in cross section, have thin walls which show various degrees of lignification, and occur in strands, around the vessels, 1 to 4 cells wide (Plate I, Figure 4). Crystal idioblasts are frequently found in their vicinity. In radial sections, paratracheal cells appear as vertically elongated cells with walls copiously pitted with simple pits. Metatracheal parenchyma cells occur in tangential bands, 2 to 7 cells wide, and, in cross section, appear to be approximately square in shape (Plate I, Figures 3—6). Crystal idioblasts are common in the metatracheal parenchyma. Cell walls are thin and show various degrees of lignification. Varying degrees of continuity of the metatracheal parenchyma bands were noted but could not be correlated with particular periods in the seasonal growth cycles as has been found by Schneider (1952) for the sweet orange in California. In radial section, metatracheal parenchyma cells appear to be vertically elongated but those containing calcium oxalate crystals (crystal idioblasts) are more cubical in shape. Continuous bands of terminal parenchyma cells are very noticeable and apparently mark the conclusion of the seasonal growth rings (Schneider 1952, Mendel 1936) (Plate I, Figures 5, 6). The cells constituting these bands, as seen in cross section, are small, squarish and have thin walls that are lignified to various degrees. As previously noted by Mendel (1936), terminal parenchyma cells greatly resemble undifferentiated cambial derivatives (Plate I, Figure 6). Conspicuously stained intracellular bodies are common in these cells.

Wood fibres. Wood fibres are very abundant and can be considered the dominant element of the wood (Webber and Fawcett 1935). In cross section, they appear to be angular (frequently approaching hexagonal) in contour. Walls are thick, heavily lignified, and cell lumina small (Plate I, Figures 3, 6). Cell size is highly variable but rarely approaches that of the wood parenchyma cells. In radial section, they appear as vertically elongated, thick walled, spindly cells of considerable length that taper gradually to a sharpish end. Walls are pitted with simple pits.

Medullary rays. As seen in cross section, the rays are both uniseriate and (more frequently) multiseriate, 2 to 6 cells wide. The uniseriate rays frequently appear as isolated strands. Ray cells are oblong, radially elongated, thin walled, and contain stored food. Cell walls show various degrees of lignification. The rays are sometimes straight, sometimes wavy, and usually deflected around the conducting vessel clusters. The end-walls of ray cells are horizontal or oblique (Plate I, Figures 3—5). In radial section, the rays are 1 to 25 cells high, individual cells being oblong, radially elongated. Under high magnification, small pits can be discerned in the cell walls.

Crystal idioblasts. Crystal idioblasts are profusely distributed throughout the various tissue systems of the xylem. Mendel's observation (1936) that crystal idioblasts are relatively rare in sweet lime rootstocks could not be confirmed. In cross section, crystal idioblasts appear as large roundish cells with irregularly thickened, highly lignified walls, in which the calcium oxalate crystal is embedded (Plate I, Figure 3). The crystal entirely fills the cell lumen and is predominantly rhomboid in shape. In radial section, the crystal idioblasts appear in vertical strands, up to 40 cells high, and are essentially squarish in shape.

Annual rings. The annual rings or seasonal growth cycles are clearly distinguishable due to the band of terminal parenchyma cells that completes them (Plate I, Figure 5). According to Schneider (1952), the formation of metatracheal wood parenchyma in sweet orange wood is influenced by the period of the growing season; no metatracheal parenchyma is formed in the early part of the grand period of growth, but at a later period occasional patches of parenchyma are laid down and, ultimately, distinct continuous bands of parenchyma appear ('terminal' parenchyma) when growth has ceased. Each annual or seasonal growth ring would thus be composed of a ring of early wood devoid of metatracheal parenchyma cells, followed by a ring of later wood characterized by the presence of bands or patches of metatracheal parenchyma, until ultimately the ring is closed by a band of terminal parenchyma. In the examined sections of Palestine sweet lime wood, there appeared to be no lack of metatracheal parenchyma in the early wood of each ring.

Cambium. In sections of samples taken in the spring (May), the cambium was found to consist of a well defined layer of cells, some 5 to 6 cells wide (Plate I, Figure 7). However, in samples taken in the winter (February) the actual cambium cells were not specifically distinguishable in the general zone of undifferentiated cells which separates the bark from the wood. In cross section, cambium cells are thin walled and tangentially elongated, sometimes hexagonal (Plate I, Figure 7). In radial section, they are vertically elongated, spindle-shaped. Conducting vessels are occasionally to be found projecting into the cambium.

The bark

Schneider (1952) has divided the secondary phloem of the sweet orange bark into 4 separate zones, each of which is characterized by a certain stage of sieve tube maturation or degeneration. The four zones are termed by him (in centrifugal order): (1) the developing phloem, (2) the functioning phloem, (3) the degenerating phloem, and (4) the non-functioning phloem. In considering the healthy anatomy of Palestine sweet lime bark, the zonal division suggested by Schneider will be adopted.

Developing phloem zone. This phloem zone was distinguishable only in samples collected in the month of January, the characteristic immature sieve tubes with folded wavy walls being evident in these sections. Fibre initials in this zone were not observed.

Functioning phloem zone. In most of the sections examined, the cambium cells lead directly into the fully mature sieve tubes characteristic of this zone (Plate I, Figure 7).

The vertical system of tissue elements is represented by sieve tubes, companion cells, parenchyma cells, crystal idioblasts, and phloem fibres. This vertical system is broken up into blocks by the horizontal phloem rays (Plate II, Figures 1, 4). The radial width of the functioning phloem zone in the sections examined ranged from 450 to 600 μ. The sieve tubes are located in radial rows, parallel to the phloem rays, and appear, in cross section, to be of irregular outline, with a large central vacuole which is usually devoid of contents (Plate II, Figure 4). The much smaller companion cell is usually found bordering one corner of the sieve tube; sieve tubes lying adjacent to the phloem rays generally have their companion cells orientated between themselves and the phloem rays. In radial section, the sieve tubes occur in vertical strands and appear as vertically elongated spindly cells that end in funnel-like structures where the sieve plates are located (Plate II, Figure 8). Their vertical length was found to be highly variable: from 60 to 200 μ in the sections examined. Parenchyma cells are numerous and interspersed amongst the sieve tubes. In this respect we agree with the observations of Schneider (1952), as opposed to those of Webber and Fawcett (1935) who reported the existence of tangential bands of phloem parenchyma cells. In cross sections, the parenchyma cells appear to be more regular and round in contour than the sieve tubes, and cellular contents are abundant (Plate II, Figure 4). In radial section, they appear as oblong, vertically elongated cells that occur in vertical strands adjoining those of the sieve tubes (Plate II, Figure 8). Crystal idioblasts are profuse and uniformly distributed throughout the functioning phloem zone. Individual idioblasts cells are large, round, with irregularly thickened walls into which is embedded the large rhomboidal calcium oxalate crystal. In radial section, they also occur in vertical strands, often 20 to 30 cells long. In most of the sections examined, the phloem fibre bundles were observed mainly in the non-functioning phloem zone and will accordingly be dealt with later. The phloem rays, in cross section, are predominantly multiseriate, 2 to 6 cells wide, though occasional uniseriate rays are to be observed (Plate II, Figure 1). In this phloem zone, the rays maintain a strict radial habit of growth. Individual ray cells are oblong, radially elongated, with end-walls which are sometimes tangential, sometimes oblique. Cellular contents (starch grains and oil droplets) are abundant, and conspicuously stained intra-cellular bodies are occasionally to be observed. In radial sections, the rays appear to be of considerable height: up to 35 cells high in the sections examined (Plate II, Figure 9).

The degenerating and non-functioning phloem zones. According to Schneider (1952), the degenerating phloem zone represents but a narrow fringe of degenerating sieve tubes (in transient stages) that emerges from the functioning phloem zone, and gradually leads into the non-functioning phloem zone characterized by the fully degenerated sieve tubes and companion cells. In the sections examined, these transient stages of sieve tube degeneration could not be observed; the zone of functioning sieve tubes leads abruptly into the zone of fully degenerated sieve tubes. As previously noted, the functioning phloem zone of the Palestine sweet lime bark is approximately 450 to 600 μ in radial width. The rest of the bark, from 4000 to 4500 μ in radial width, is taken up by

the tissues of the non-functioning phloem zone and periderm — the outermost part of the non-functioning phloem zone serving as cortical tissue in citrus bark (Schneider 1952). The non-functioning phloem zone is characterized by the general distortion of its constituent tissue elements; crushed sieve tubes and companion cells, crushed and normal parenchyma cells, altered phloem rays, phloem fibre bundles, sclereids, and crystal idioblasts (Plate II, Figures 2, 3, 5). When viewed under low magnification, cross sections show that the phloem rays, in continuing their approximate radial habit of growth, enclose between them blocks of crushed and flattened sieve tubes and companion cells, occasional crushed parenchyma cells, and unaltered crystal idioblasts (Plate II, Figures 2, 5). This radial system is broken up by the tangential bands of phloem fibres. Towards the periderm, the phloem rays flare out into wide masses of parenchymatous tissue that frequently contain sclereids (Plate II, Figures 2, 3). These large masses of parenchymatous tissue eventually join up tangentially and form a continuous band of parenchymatous cortical tissue that lies directly underneath the periderm. Sclereids are abundant in this area (Plate II, Figure 3). In cross section, the crushed sieve tubes appear as tangentially stretched, collapsed cells, with wavy, inwardly folded walls. Interspersed between them are non-crushed parenchyma cells (Plate II, Figure 5). Approaching the periderm, the crushed sieve tubes are further compacted, cell lumina are obliterated, and a compact mass of folded and flattened cell walls results (Plate II, Figure 3). The crushed tubes are discoloured brownish-yellow. In radial section, the crushed sieve tubes and companion cells appear as wavy, vertically elongated, strands of long spindly cells with narrow lumina, which lie between strands of normal parenchyma cells and crystal idioblasts (Plate II, Figure 9). It is to be noted that many of the parenchyma cells in the non-functioning phloem zone persist in an unaltered state and lie interspersed between the folded walls of the crushed sieve tubes (Plate II, figure 5). Similarly, the crystal idioblasts, which occur in profuse quantities throughout this zone, do not undergo crushing or other alterations. Particularly large concentrations of idioblasts are to be found surrounding the phloem fibre bundles (Plate II, Figure 6). The histological picture of the crystal idioblasts is similar to that described for the functioning phloem zone. As previously noted, the phloem fibre bundles occur mainly in the non-functioning phloem zone and are located in tangential bands, though occasional isolated clusters of fibres are to be observed near the periderm (Plate IV, Figure 1). In the sections examined, from 2 to 8 tangential bands of fibres were observed. Thus we could not confirm the observation of Mendel (1936) that Palestine sweet lime bark is characterized by only one layer of phloem fibre bundles. Each bundle is made up of some 20 to 300 closely packed phloem fibres (Plate II, Figure 6). In cross section, phloem fibres appear to have thick, highly lignified walls, cell lumina being practically obliterated. Individual fibre diameters ranged from 6 to 27 μ. In radial section, fibres occur in vertical strands of varying length (individual fibres up to 800 μ in length were recorded). Cell lumina are almost obliterated and fibres taper to a sharp point. Sclereids are of frequent occurrence in the masses of ray-derived parenchymatous tissue that assumes the function of cortical tissue (Plate II, Figure 3). The sclereids occur in tangentially orientated

bundles — some 8 to 22 sclereids per bundle (Plate II, Figure 7). Each individual sclereid is essentially a tangentially elongated cell, as seen in cross section, with thick, heavily lignified, walls which are copiously pitted with a peculiar 'branching-canal' type of pitting. This type of wall pitting is considered characteristic of sclereids (Eames and MacDaniels 1947). Sclereids are also occasionally found, in cross section, to be of roundish contour. In radial section, sclereids are predominantly round in outline and the characteristic 'branching canal' type of pitting that radially spreads out from the nearly obliterated cell lumina is particularly striking.

From the above description it will be seen that the cortical tissue in Palestine sweet lime bark in composed of a matrix of tangentially elongated ray-derived parenchyma cells, through which the crushed and folded walls of degenerated sieve tubes interweave, and into which are embedded groups of sclereids (outermost part) or phloem fibre bundles (innermost part) (Plate II, Figure 3).

Periderm. The different layers comprising the periderm in Valencia sweet orange bark have been extensively dealt with by Webber and Fawcett (1935) and no deviations from their description have been noted in the bark of the Palestine sweet lime.

Epidermis. In the sections examined by us, remnants of the epidermis were not observed.

PATHOLOGICAL MORPHOLOGY

The xyloporosis disorder is characterized, as previously noted, by thorn-like projections or pegs on the inner face of the bark which fit into corresponding conoid shaped pits on the cambial face of the wood. The morphology of these deformations in the mature, diseased, Palestine sweet lime rootstocks examined by us may be summarized as follows: Thorn-like projections or pegs on the cambial face of the bark, with vertically elongated oblong bases, which range from 2—8 mm in length. The pointed apices of the pegs are occasionally brownish in colour. The horizontal depth of the pegs varies from 1 to 6 mm. Corresponding conoid pits on the cambial face of the wood are oval in outline, with long axis parallel to grain of wood, and range from 2 to 8 mm in length. Pits are occasionally brownish in colour, and the peg and pit deformations are massed just below the bud-union (Plate I, Figure 2). The above description is similar to that of the "conoid-type" pitting form published by DuCharme and Knorr (1954).

In addition to the peg and pit abnormalities, involving the cambial face of both wood and bark, small shallow depressions were frequently observed on the outer bark face of young diseased rootstocks; these outer bark depressions correspond with the inner peg and pit deformations. The depressions are roundish to oval in outline, are surrounded by a raised ridge of bark, and have slightly raised centres in which a small cavity can be seen (Plate I, Figure 1). This small cavity, as will be seen later, corresponds exactly with the tip of the thorn-like projection or peg on the inner bark face. In the samples examined, these outer bark depressions measured from 3 to 35 mm in diameter. The outer bark depressions were only observed in young diseased rootstocks; the external bark face of older diseased rootstocks was normal.

PATHOLOGICAL ANATOMY AND HISTOLOGY

The cross and radial sections of the affected bark and wood were cut, as far as was possible, through the central axis of the phloem peg and xylem pit deformation (if the phloem peg is likened to a pyramid, then the 'central axis' is the line drawn between the apex of the pyramid and the centre of its base). It should be noted that the histological structure of the peg and pit deformation varies according to the place, above or below the central axis, at which the sections were cut. A series of sections were also taken through complete pegs in order to obtain a fuller picture of their internal structure. However, unless otherwise stated, the following anatomical descriptions apply to the sections cut through the central axis of the peg and pit deformation.

A cross section through a peg and pit deformation reveals a triangular mass of phloem tissue that is wedged into the xylem (Plate IV, Figure 2). This abnormality is accompanied by irregularities in the new wood adjoining the wedged-in phloem peg, in the wood of previous seasonal growth rings, and in the bark.

Irregularities in the wood

Irregularities in the wood adjacent to the peg-pit deformation. The young xylem tissues adjacent to the wedged-in phloem peg show considerable distortion. Although all the constituent elements of normal xylem tissue are present here, some of these elements are apparently orientated in abnormal planes (Plate III, Figures 1, 2, 3). As seen in cross section, the wood fibres have lost their typical angular outline and have become stretched and elongated in various tangential directions (Plate III, Figures 2, 6). Some xylem vessels and parenchyma cells also show this tangential distortion (Plate III, Figures 2, 4). Medullary ray cells and crystal idioblasts retain their normal habit of growth; the distortion thus involves only the block of xylem vessels, fibres, and parenchyma cells enclosed by the normal rays (Plate II, Figure 2). The degree of distortion in the young xylem tissues appears to vary according to the magnitude of the peg-pit deformation; in large pegs which penetrate deep into the wood, a radial band of highly distorted xylem tissue, which connects up with lesions in the wood of previous seasonal growth rings, can frequently be observed. In some of the 4 year old affected rootstocks, in which the anatomical manifestation of the disease was to be found in the wood of only two consecutive growth rings, the distortion of the xylem tissues adjacent to the phloem peg formation had resulted in almost complete obliteration of the conducting vessels in that area. The highest degree of tissue distortion is to be found at the central axis of the peg-pit deformation; further away from the central axis, on all sides, the distortion becomes progressively less.

Radial sections of the distorted xylem tissues show that the xylem vessels, wood fibres, and parenchyma cells are inwardly bent and kinked in a wavy fashion (Plate III, Figure 3). The conducting vessels in this distorted wood also appear to be narrower, shorter, and fewer in number than is usual in normal wood.

Irregularities in the wood of previous seasonal growth rings. Cross sections of the diseased trunk show numerous lesions in the wood of previous seasonal growth rings. These lesions are predominantly triangular in shape, and are bordered on their non-cambial side by terminal parenchyma cells. The bands of terminal parenchyma cells are accordingly bent inwards at these places (Plate III, Figures 1, 4) so that the seasonal growth rings appear to be kinked — a phenomenon that has been previously reported by Reichert and Perlberger (1934). Lesions occur either at isolated spots, adjoining the band of terminal parenchyma cells, or in an almost continuous tangential band that is interrupted by only brief patches of normal terminal parenchyma cells. The inner wood lesions are bordered on their cambial side by distorted xylem tissue similar to that described in the previous section.

Where lesions occur in the wood of several consecutive growth rings, they are usually to be found in radial alignment; the lesions in the wood of one particular growth ring being opposite those in the adjacent ring. In such cases, the tissue between the lesions in the two adjacent growth rings shows the typical tangential distortion previously described. Lesions in the second last seasonal growth ring are frequently connected with peg and pit deformations at the cambium by a radial band of tangentially distorted xylem tissue lying between normal medullary rays (Plate III, Figure 1).

Radial sections of some samples of affected wood show that the inner wood lesions are often of considerable vertical height and are frequently in vertical alignment, one on top of the other. Furthermore, the conducting vessel members in the inter-lesion xylem tissue are bent, narrow, and few in number (Plate III, Figure 3).

In the samples taken from the older affected rootstocks, the inner wood lesions were found to measure from 0.1 to 0.8 mm in tangential length, 0.1 to 2 mm in radial width, and up to 4.8 mm in vertical height. In samples taken from the young 4 year old diseased rootstocks, the lesions were considerably larger: up to 5 mm in tangential length, up to 8 mm in radial width, and up to 12 mm in vertical height.

The histological structure of the inner wood lesions has also been studied. They are composed of large, approximately isodiametric, wood-parenchyma-like cells. In cross-section, these cells are sometimes irregularly angular in outline (Plate III, Figure 5). Cell walls are thin and occasionally lignified to various degrees; in the latter case minute pits can frequently be observed in the slightly lignified walls. Large, conspicuously stained, intracellular bodies are common in these wound parenchyma cells (Plate III, Figure 5). Numerous crystal idioblasts are to be found distributed throughout the lesion. The terminal parenchyma cells, bordering the lesion on its non-cambial side, appear to be somewhat less lignified than is usual in normal wood. In outline they are frequently kidney-shaped (Plate III, Figure 7), whereas in normal wood they are invariably squarish (Plate I, Figure 6).

The medullary rays are usually deflected around the smaller lesions, but may sometimes appear to be broken off at the larger lesions. In these latter instances, it is thought that large hypertrophied ray cells may also occur amongst the wood parenchyma cells which constitute the lesion. However, this point could not be established with

any finality. The medullary ray cells, interrupting the inter-lesion patches of terminal parenchyma, frequently show hypertrophy; the cells lose their typical brick-like form and become larger and more squarish in outline.

Gum impregnation tests. In the samples of wood taken from the diseased 17 year old rootstocks, little gum impregnation of the tissues was observed without the aid of a wound gum reagent. However, when a saturated solution of phloroglucin in 18% HCl was applied to fresh sections of the affected wood, positive reactions, indicating the presence of wound gum, were observed in the xylem tissue surrounding the inner wood lesions, and also in the distorted tissue of the newer wood adjacent to the phloem peg at the cambium. Non-distorted tissue further away from the cambial peg-pit deformation gave a negative reaction. In sections cut from the wood of diseased 4 year old rootstocks, gum impregnation was conspicuous without the aid of a reagent and involved the wound parenchyma cells of the inner wood lesions, the surrounding distorted xylem tissue, and the young xylem tissue adjacent to the peg deformation at the cambium.

It is to be noted that in all the sections examined, gumming up of the conducting vessel lumina was observed in isolated instances only (Plate III, Figure 7).

Irregularities in the bark

Irregularities associated with the phloem peg deformation. The tissue elements comprising the peg are: (1) abnormally expanded phloem rays, whose cells have undergone varying degrees of hyperplasy and hypertrophy; (2) narrow radial rows of crushed, necrotic sieve tubes and companion cells lying between the abnormal phloem rays; (3) occasional groups of hypertrophied parenchyma cells; (4) phloem fibre bundles; and (5) crystal idioblasts. The entire inter-ray tissue is almost obliterated by the hyperplastic phloem rays, which may be considered the dominant element of the peg. Conspicuously stained intracellular bodies are frequently found in the abnormal ray cells. Many of the crushed sieve tubes show callus formation (sieve plates are stained bright blue by lacmoid stain). Parenchyma cells in the inter-ray tissue are normal, crushed, or hypertrophied. The hypertrophied parenchyma cells are mainly evident at the base (towards the periderm) of the peg (Plate IV, Figures 3, 5, 6). Crystal idioblasts, in an unaltered state, are profusely distributed throughout the tissues of the peg. The extreme tip of the peg (at the cambium) is almost entirely composed of hypertrophied and hyperplastic ray cells that join up with an apparently normal medullary ray in the outer xylem. In the numerous sections examined, it was observed that the cambium cells persist and fully line the phloem peg deformation. Some distortion of the cambium cells was, however, encountered. Clusters of phloem fibre bundles are common inside the peg and probably impart to it mechanical strength. Cross sections of the affected bark show, under low magnification, that the tangential bands of phloem fibre bundles tend to follow the contour of the cambium and thus appear to be deflected into the peg (Plate IV, Figure 1). This deflection involves mainly the tangential bands of phloem fibres in the functioning and inner part of the non-functioning phloem zone; those of the outer bark remain straight as in normal bark.

The phloem rays of the affected bark show a tendency to converge into the peg deformation. The cells of such rays show hypertrophy and hyperplasy which has resulted in a crushed inter-ray tissue of sieve tubes, companion cells, and parenchyma cells, similar to that inside the peg deformation. This crushed tissue appears as an extension of the non-functioning phloem zone, so that the radial width of the functioning phloem zone progressively decreases until functioning phloem tissue disappears altogether (Plate IV, Figure 1). Radial sections of the affected bark also reveal extensive phloem necrosis both inside the peg and in the adjacent functioning phloem zone. It is to be noted, however, that sections taken above and below the central axis of the peg deformation show that some functioning phloem tissue persists even inside the peg.

No abnormalities in the outer non-functioning phloem zone (cortex) or periderm are encountered in the older diseased rootstocks. Cross sections of the bark of the 4 year old diseased rootstocks, however, show that the cortex and periderm tend to follow the same contour as the cambium lining the phloem peg deformation. Thus, on the same axis as the central axis of the peg, the cortex and periderm are kinked inwards to form a small triangular cavity (Plate IV, Figure 4). This cavity corresponds to the small hole, previously described in the section on morphology, in the centre of the oval depressions appearing on the outer bark face of the young diseased stocks (Plate I, Figure 1). The kinked periderm and cortex tissue are heavily impregnated with gum. The histopathology of the phloem peg deformation in the young rootstocks is similar to that of the older rootstocks. It should once again be stressed that this abnormality of the outer bark was evident only in the 4 year old affected rootstocks; bark sections taken of 6 and 7 year old diseased rootstocks showed the outer bark tissues to be normal. This was also confirmed by our macroscopic observations in the orange grove.

Bark irregularities not associated with the phloem peg deformations. Cross and radial sections of affected bark were examined in order to determine whether sieve tube necrosis in the functioning phloem zone is to be found only in association with the phloem peg deformations. In a number of instances, abnormal islands of necrotic sieve tubes and companion cells were observed in the functioning phloem zone, as distinct from those associated with peg deformations (Plate IV, Figure 7). Radial sections also confirmed this point. It is assumed that these instances of phloem necrosis result from the causal agent of xyloporosis; though the possibility always exists that the Palestine sweet lime rootstocks sampled were infected with another virus, in addition to xyloporosis, that causes phloem necrosis.

Gum impregnation of the affected bark. Gum impregnation of the diseased bark tissues of older rootstocks is not always evident without the use of a reagent. However, a saturated solution of phloroglucin in 18% HCl applied to fresh sections of the affected bark shows the presence of wound gum in the crushed and necrotic sieve tubes inside the peg deformation. Islands of gum impregnation also appear in the functioning phloem zone adjacent to the peg. Moreover, the non-functioning phloem zone of the diseased bark as a whole shows a strong reaction (in comparison to healthy bark)

indicating the presence of considerable quantities of infiltrated gum. Under higher magnification, it appears that the infiltrated gum in this zone is confined to the necrotic sieve tubes and companion cells. When the wound gum reagent was applied to longitudinal sections cut across the bud-union of the diseased Shamouti on Palestine sweet lime rootstock trees, it was observed that the gum impregnation of the rootstock bark tissues did not extend into the Shamouti scion tissues, but abruptly ceased at the bud-union. It is interesting to note that a similar gum impregnation of necrotic sieve tubes, which is revealed by the application of phloroglucin in HCl, has also been observed by Schneider (1945) in the bark of peach and cherry trees affected with the Buckskin virus disease.

The bark tissues of the affected 4 year old rootstocks are conspicuously impregnated with gum and no recourse to a wound gum reagent is necessary. The gum impregnation in these bark samples is concentrated around the phloem peg deformation and adjacent tissues.

DISCUSSION

The first point to be considered is the possible relationship between the anatomical abnormalities and the deterioration of the tree. Our observations have shown that lesions of considerable size are common in the wood of previous years' growth rings; in the young affected rootstocks, these lesions measured up to 5 mm in tangential length, 8 mm in radial width, and 12 mm in vertical height. The inner wood lesions are also, in both the young and mature affected rootstocks, orientated in radial, tangential, and vertical alignment inside the woody cylinder of the tree. It has also been noted that the distorted xylem tissue, adjacent to the inner wood lesions and pit-peg deformations, is frequently characterized by abnormal conducting vessels. We are therefore inclined to assume that these abnormalities will significantly limit the extent of wood tissue effective in water transport, and, accordingly, the water conducting capacity of the diseased rootstocks. Furthermore, these wood alterations are of greatest frequency just underneath the bud-union — a location where, even in the most ideally budded trees, the upward movement of water and minerals from the roots is likely to be somewhat obstructed. In the older affected rootstocks, in which the anatomical manifestation of the disease has been present for a considerable number of years, the accumulative effect of these wood abnormalities might well be responsible for the slow decline of the trees. Since gumming of xylem vessel lumina was observed in relatively few instances, we also tend to conclude that gum-plugging of the xylem vessels does not play an important part in the deterioration of the tree; in this respect, the wood alterations induced by xyloporosis are distinct from those induced by the psorosis virus (Webber and Fawcett 1935). Virus induced degenerative changes in the xylem have also been noted in Pierce's disease of grape vines and the Phony disease of peach trees (Esau 1948).

In a first report on xyloporosis, Reichert and Perlberger (1934) attributed the deterioration of the tree to injury sustained by the sieve tubes in the phloem tissues associated with the peg deformations. This supposition was supported by quantitative sugar

analysis of the juice of affected tree fruits: the percentage of total soluble solids in the fruit juices of affected trees was higher than in fruits of healthy trees. The above authors concluded that accumulation of photosynthetic products in the leaves of the upper parts of the tree would decrease the photosynthetic activity of these leaves. Our observations have also shown that extensive sieve tube necrosis occurs in the phloem tissues comprising the peg deformation, in the functioning phloem tissues adjacent to the peg, and also in the functioning phloem tissues not associated with peg deformations (although in this latter case, as previously noted, the sieve tube necrosis may result from another virus infection). It would seem, therefore, that in places where the bark deformations are of great frequency, the extensive sieve tube necrosis might seriously hamper the downward movement of photosynthetic products to the roots and effect the consequent degeneration of the root system. Virus induced sieve tube necrosis, in the neighbourhood of the bud-union, is well known in other virus diseases of fruit trees (Schneider 1945, 1954).

The anatomical observations have thus shown that the abnormalities in both bark and wood are capable of causing the decline of the tree. The relationship between the rootstock wood alterations and the foliage decline of the tree top would be further confirmed by an examination of the physical water conducting ability of portions of xyloporosis affected rootstocks in comparison to healthy stocks; a similar relationship in psorosis of citrus has been clarified in this manner (Bitancourt, Fawcett and Wallace 1943). A study of the starch relationships, throughout the growing season, of healthy and xyloporosis affected rootstocks would be of value in determining the degree of tree deterioration which results from sieve tube necrosis in the bark.

In view of the fact that this anatomical study of xyloporosis diseased tissues has been confined to the static aspect only, and no study made of the developmental anatomy of peg and pit deformations, we feel that any discussion on the genesis of these bark and wood alterations must be accordingly limited. Reichert and Perlberger (1934), in their theory on the mode of formation of the peg-pit deformations, have suggested a disintegration and inactivation of the cambium cells at a certain localized spot, and that on all sides of this spot the rate of formation of new cambial derivatives increases proportionately to the distance from the affected spot. Thus the peg-pit deformation is conoid in shape; the innermost part of the pit, and corresponding tip of the peg, corresponds to the actual diseased spot in the cambium where there was complete inactivation of secondary growth. Furthermore, according to the above authors, the inner wood lesions represent the peg and pit deformations of previous years. Our observations have, in a sense, confirmed this point since we have shown that the degree of tissue alteration decreases as the distance from the central axis of the peg-pit deformation increases. But we have also shown that the tissues comprising the peg are composed of necrotic sieve tubes and companion cells lying between hypertrophied and hyperplastic phloem rays. This appears to indicate that, in the formation of the peg, another set of factors operates and results in abnormal expansion of the phloem rays and consequent crushing of the inter-ray phloem tissue. It should also be noted that both Reichert and Perl-

berger (1934) in connection with xyloporosis, and Hughes and Lister (1953) in connection with die-back disease of limes in the Gold Coast, have observed disintegrated cells impregnated with gum at the cambium (leading to the formation of gumpockets, according to Hughes and Lister). Such abnormalities were not observed by us. A possible reason for this might be that cellular disintegration and gumming at the cambium may occur only at certain times of the growing season. A similar condition has been reported by Fawcett and Bitancourt (1943) in psorosis of citrus; these authors have found that, at certain periods of growth, conditions do not favour the formation of gum pockets at the cambium.

The disorted xylem tissues adjacent to the cambium peg-pit deformations and inner wood lesions, probably arises from changed planes of division in the cambium. The inactivation of cambial activity at the diseased spots, and consequent unequal rates of secondary growth, would result in altered pressure conditions acting at the cambium. Such altered pressure conditions would bring about the changed planes of division of the xylem cambial derivatives (Webber and Fawcett 1934).

The study of the outer bark anatomy of the young diseased rootstocks has shown that the triangular cavity, in the centre of the small depression, appears to be intimately associated with the peg-pit deformation on the inner face of the bark. This cavity probably arises due to the cessation of secondary growth at the affected spot in the cambium. We have also noted that, as the affected rootstock matures, this outer bark abnormality disappears. The explanation of this might well be that, as the affected rootstock increases in girth, the piling up of the old non-functioning phloem layers in the cortex will tend to obscure, and ultimately conceal, this outer bark abnormality. Our observations on the outer bark of mature affected rootstocks tend to confirm this suggestion. Should further field surveys indicate this outer bark abnormality to be a consistent symptom-expression, it would then enable the grower to examine his young budlings for xyloporosis pitting symptoms without first removing a patch of bark. The established practice of cutting out a portion of bark in order to determine whether pitting occurs on the cambial face of the wood, is not only detrimental, by its girdling action, to the young budling, but is also inconclusive since it exposes only a limited part of the wood for examination.

ACKNOWLEDGMENTS

The author wishes to express his thanks to Professor I. Reichert, who suggested this work and under whose guidance and encouragement it was carried out. He is also indebted to Drs. I. Wahl and K. Mendel for their kind assistance.

REFERENCES

1. Batchelor, L. D. and Webber, H. J., 1948, *The Citrus Industry*, vol. 2, p.100, University of California Press, Berkeley.

2. Bitancourt, A. A., Fawcett, H. S. and Wallace, J. M., 1943, The relations of wood alterations in psorosis of citrus to tree deterioration, *Phytopathology*, **33**, 865—883.

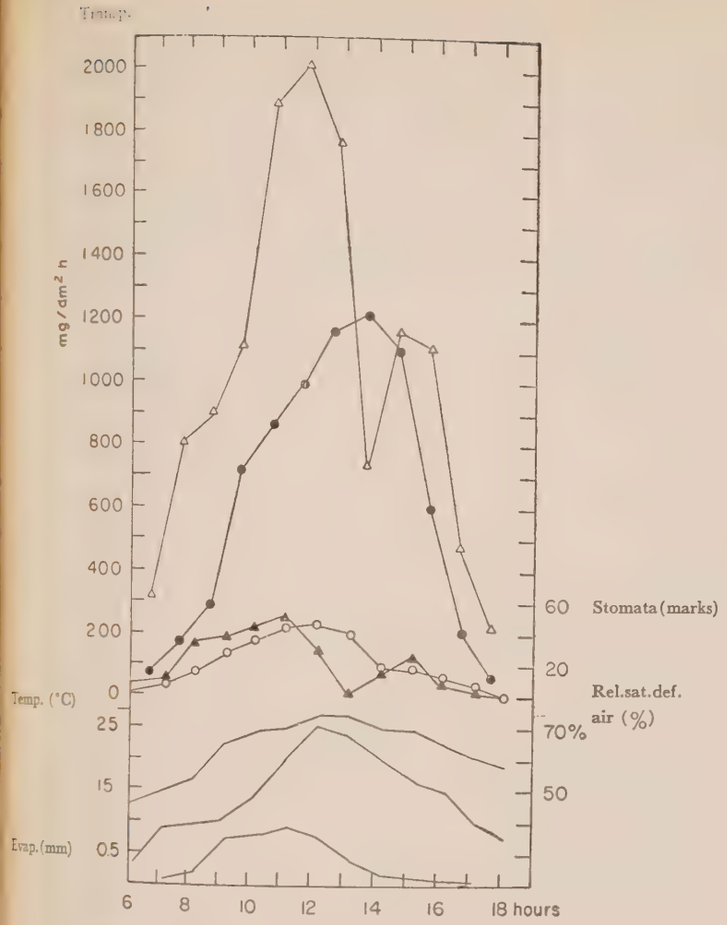


FIGURE 1. Transpiration of mature Shamouti orange leaves, first Sharav day (May 4, 1953).

Transpiration Δ——Δ of sun leaves (mg/dm².h)
●——● of shade leaves (mg/dm².h)
Stomatal aperture ⧋——⧋ of sun leaves (marks)
O——O of shade leaves (marks)

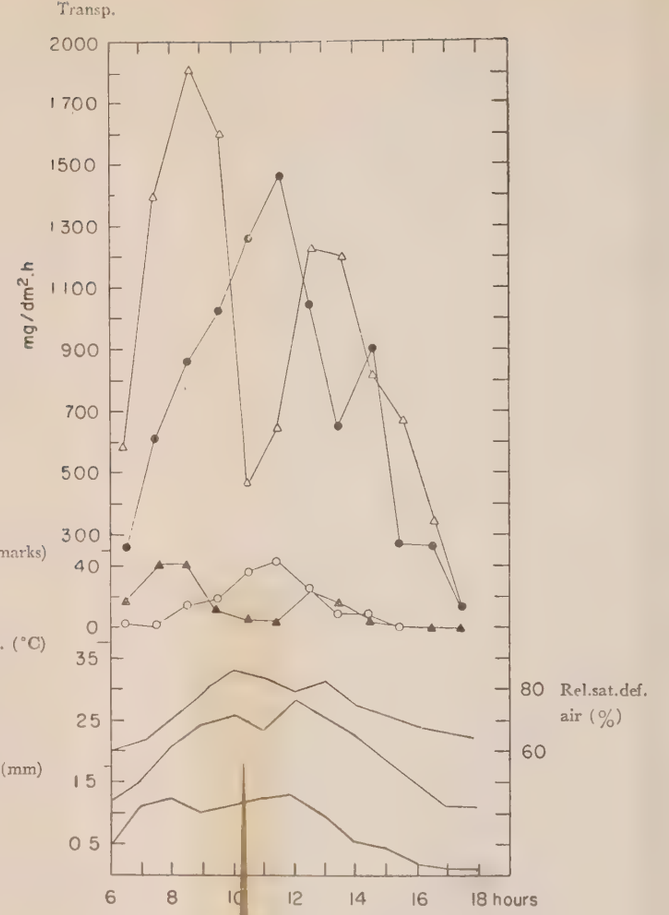


FIGURE 2. Transpiration of mature Shamouti orange leaves, second sharav day (May 5, 1953).

Transpiration Δ——Δ of sun leaves
●——● of shade leaves
Stomatal aperture ⧋——⧋ of sun leaves
O——O of shade leaves

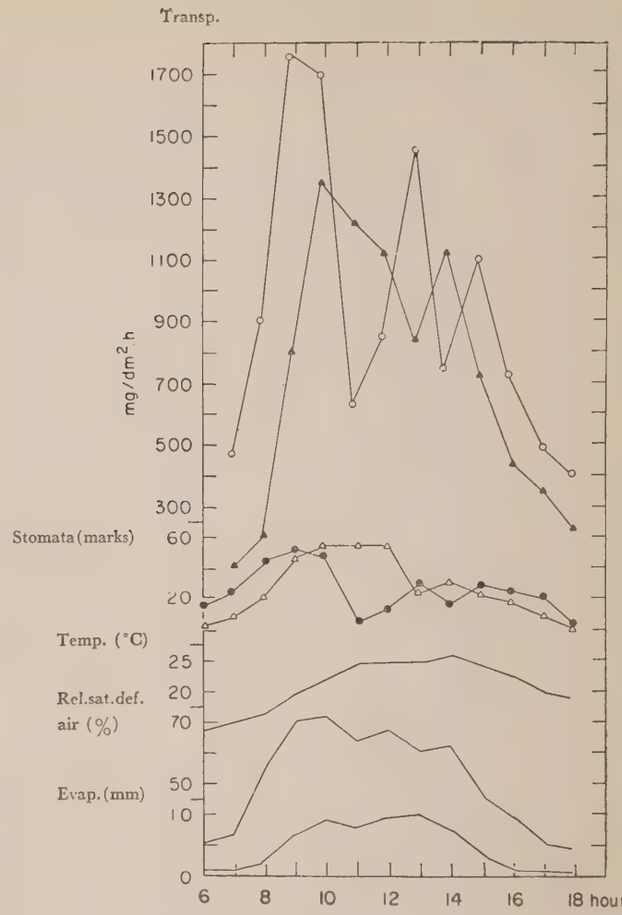


FIGURE 3. Transpiration of mature Shamouti orange leaves, second Sharav day (April 24, 1953).

Transpiration O——O of sun leaves
⧋——⧋ of shade leaves
Stomatal aperture ●——● of sun leaves
Δ——Δ of shade leaves

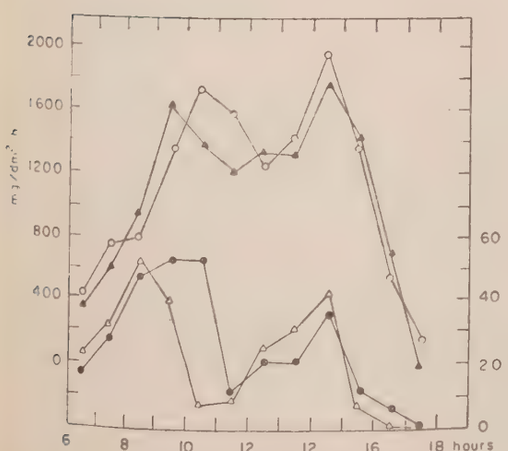


FIGURE 4. Transpiration of young and mature Shamouti orange sun leaves (8.5.1953).

Transpiration O——O of mature leaves
⧋——⧋ of young leaves
Stomatal aperture Δ——Δ of mature leaves (marks)
●——● of young leaves (marks)

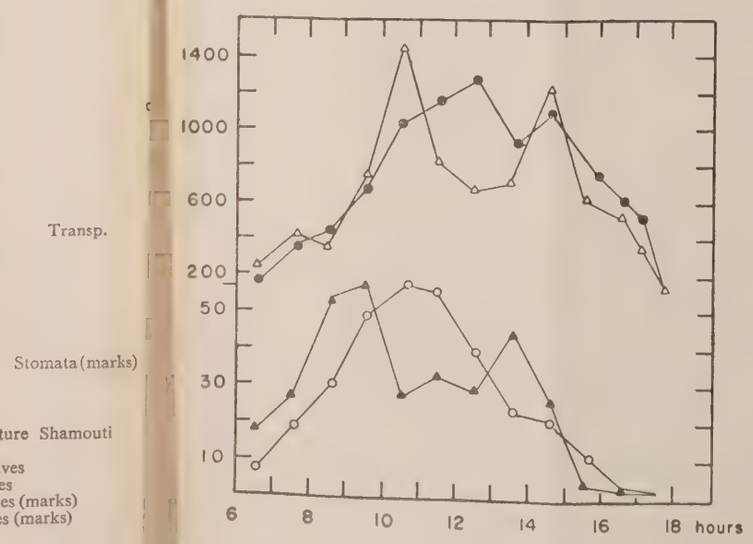


FIGURE. 5 Transpiration of young and mature Shamouti orange shade leaves (May 8, 1953).

Transpiration ●——● of mature leaves (mg/dm².h)
Δ——Δ of young leaves
Stomatal aperture O——O of mature leaves (marks)
⧋——⧋ of young leaves (marks)

3. Goldschmidt-Blumental, S., 1956, The morphology and anatomy of the bud union of Shamouti on various stocks, *Bull. Res. Counc. of Israel*, **5D**, 143—154.

4. Childs, J. F. L., 1952, Cachexia disease, its bud transmission, and relation to xyloporosis and tristeza, *Phytopathology*, **42**, 265—268.

5. Costa, A. S., Grant, T. J. and Moreira, S., 1950, A possible relationship between tristeza and the stem pitting disease of grapefruit in Africa, *Calif. Citrogr.*, **35**, 504, 526—528.

6. DuCharme, E. P., 1952, Xyloporosis of citrus, *Citrus Mag.*, **14**, 25.

7. DuCharme, E. P. and Knorr, L. C., 1954, Vascular pits and pegs associated with diseases in citrus, *Plant Dis. Reptr*, **38**, 127—142.

8. Du Charme, E. P. and Suit, R. F., 1951, Xyloporosis of citrus in Florida, *Plant Dis. Reptr*, **35**, 556—557.

9. Eames, A. J. and MacDaniels, L. H., 1947, *An Introduction to Plant Anatomy*, p. 88, McGraw-Hill Book Co., New York.

10. Esau, K., 1948, Anatomic effects of the viruses of Pierce's disease and Phoney Peach, *Hilgardia*, **18**, 423—481.

11. Fawcett, H. S., 1937, Contacts with the citrus industry and other observations in South America, *Calif. Citrogr.*, **22**, 552—553, 571—572, 575.

12. Fawcett, H. S. and Bitancourt, A. A., 1943, Comparative symptomology of psorosis varieties on citrus in California, *Phytopathology*, **33**, 837—864.

13. Hughes, W. A. and Lister, C. A., 1953, Lime die-back in the Gold Coast, a virus disease of the lime, *Citrus aurantifolia* (Christmann) Swingle, *J. hort. Sci.*, **28**, 131—140.

14. Johansen, D. A., 1940, *Plant Microtechnique*, McGraw-Hill Book Co., New York.

15. McClean, A. P. D., 1950, Possible identity of three citrus diseases, *Nature, Lond.*, **165**, 767—768.

16. Mendel, K., 1935/36, The anatomy and histology of the bud-union in citrus, *Palest. J. Bot. hort. Sci.*, **1**, 13—46.

17. Rawlins, T. E., 1933, *Phytopathological and Botanical Research Methods*, p. 53, John Wiley and Sons, New York.

18. Reichert, I., 1953, Xyloporosis in citrus, *Proc. XIII Int. hort. Congr.*, London, 1275—1280.

19. Reichert, I., 1954, Our knowledge of xyloporosis in citrus, *Rapp. et Comm. VIII Int. bot. Congr.*, Paris, 164.

20. Reichert, I. and Perlberger, J., 1934, Xyloporosis, the new citrus disease, *Hadar*, **7**, 163—167, 172, 183—202.

21. Reichert, I., Yoffe, I. and Bental, A., 1953, Shamouti orange on various rootstocks and its relation to xyloporosis, *Palest. J. Bot. Rehovot*, **7**, 163—184.

22. Sass, J. E., 1940, *Elements of Botanical Microtechnique*, p. 61, McGraw-Hill Book Co., New York.

23. Schneider, H., 1945, Anatomy of buckskin-diseased peach and cherry, *Phytopathology*, **35**, 610—635.

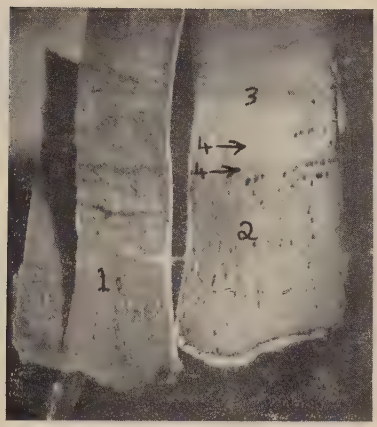
24. Schneider, H., 1952, The phloem of the sweet orange tree trunk and the seasonal production of xylem and phloem, *Hilgardia*, **21**, 331—366.

25. Schneider, H., 1954, Anatomy of bark of bud-union, trunk, and roots of quick-decline-affected sweet orange trees and sour orange rootstock, *Hilgardia*, **22**, 567—581.

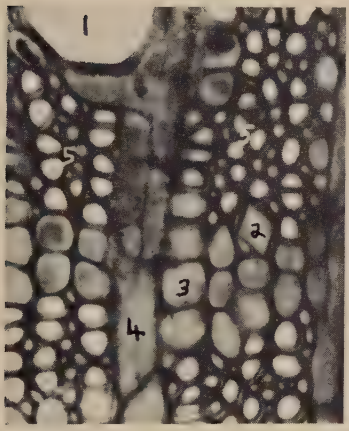
26. WALLACE, J. M., 1951, Recent developments in studies of quick decline and related diseases, *Phytopathology*, **41**, 786—793.

27. WEBBER, I. E. and FAWCETT, H. S., 1935, Comparative histology of healthy and psorosis-affected tissues of *Citrus sinensis*, *Hilgardia*, **9**, 71—109.

1



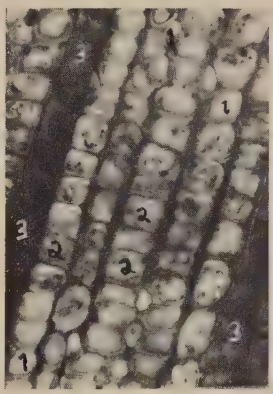
2



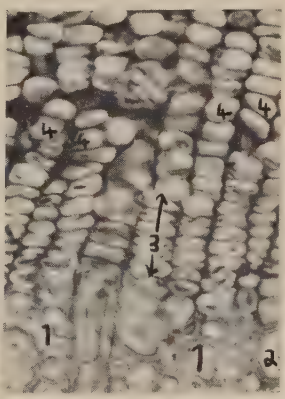
3

4

5

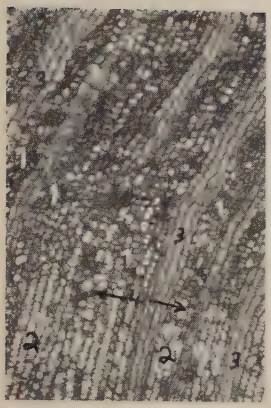


6

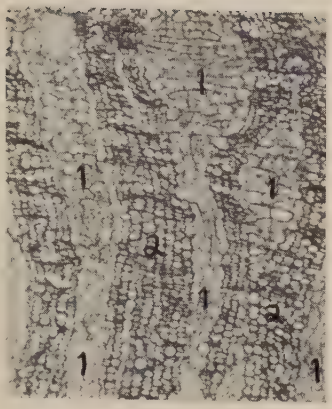


7

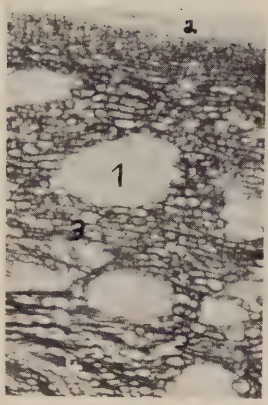
Plate I. 1. Outer bark face of young xyloporosis affected Palestine sweet lime rootstock. Note the ovoid shaped depression. surrounded by raised lip in the centre of which is a small cavity (1). (×3). 2. Portion of trunk of Shamouti budded to Palestine sweet lime rootstock. The bark is turned around in order to show pitting in wood and corresponding pegs on bark. (1) Rootstock bark showing pegs; (2) rootstock wood showing pits; (3) Shamouti scion wood; (4) bud-union. 3. Cross section of healthy rootstock wood. (×400). (1) Conducting vessel; (2) crystal idioblast; (3) metatracheal parenchyma cell; (4) medullary ray; (5) wood fibres. 4. Cross section of healthy wood showing arrangement of tissues. (× 80). (1) Conducting vessel; (2) metatracheal parenchyma cells; (3) medullary rays; (4) paratracheal parenchyma cells; (5) wood fibres. 5. Cross section of healthy wood showing band of terminal parenchyma cells (1) that indicates the completion of a growth ring. (× 25). 6. Cross section of part of terminal parenchyma band shown in Figure 5. (× 400). (1) Wood fibres; (2) terminal parenchyma cells; (3) medullary ray cells. 7. Cross section through healthy wood and bark showing cambium. (× 400). (1) Wood fibres; (2) conducting vessel; (3) cambium cells; (4) sieve tubes and companion cells.



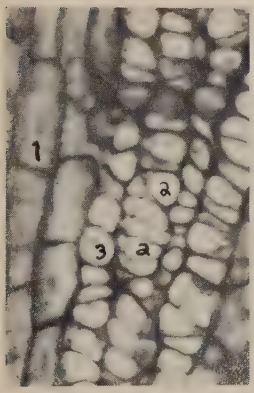
1



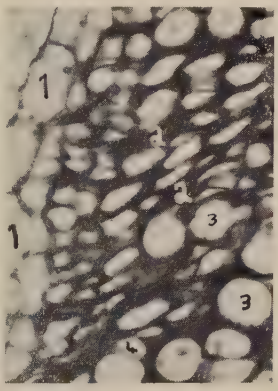
2



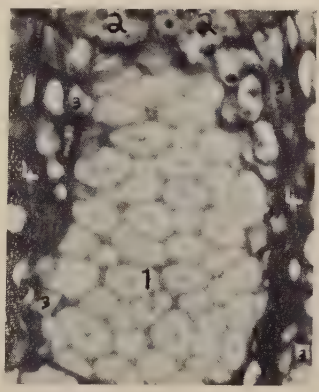
3



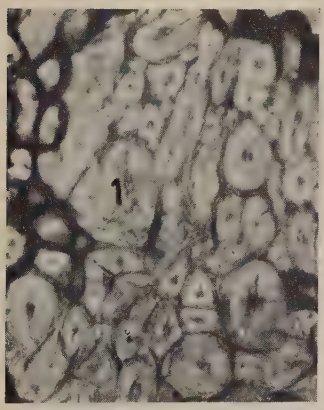
4



5

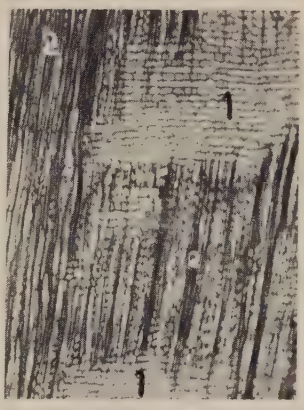


6



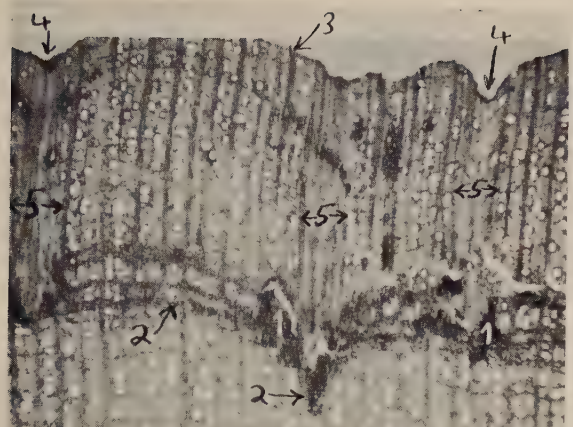
7

8

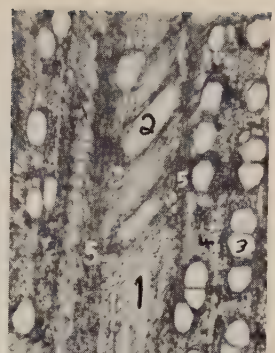


9

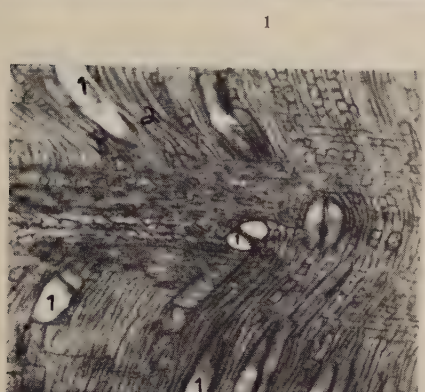
Plate II. Healthy bark. 1. Cross section showing part of the functioning and non-functioning phloem zones. Cambium towards bottom. (× 80). (1) Necrotic sieve tubes in non-functioning phloem zone; (2) normal sieve tubes in functioning phloem zone; (3) phloem rays; (4) boundary between functioning and non-functioning phloem zones. 2. Cross section of non-functioning phloem zone. Note flare-out of phloem rays. (× 80). (1) Phloem rays; (2) necrotic sieve tubes and unaltered parenchyma cells. 3. Cross section showing cortical tissue of bark. (× 80). (1) Sclereids; (2) periderm; (3) ray-derived parenchymatous tissue; (4) crushed sieve tubes. 4. Cross section of part of functioning phloem zone shown in Figure 1. (× 400). (1) Phloem ray cells; (2) sieve tubes and companion cells; (3) parenchyma cells. 5. Cross section of part of non-functioning phloem zone shown in Figure 1. (× 400). (1) Ray cell; (2) crushed sieve tubes and companion cells; (3) unaltered parenchyma cells; (4) crystal idioblasts. 6. Cross section showing phloem fibre bundles. (× 400). (1) Phloem fibres; (2) ray cells; (3) crystal idioblasts; (4) crushed sieve tubes. 7. Cross section showing circular sclereids (1). Note the peculiar "branching-canal" type of pitting. (× 400). 8. Radial section showing sieve tubes. (× 400). (1) Parenchyma cell; (2) sieve tube; (3) sieve plate; (4) ray cell. 9. Radial section showing non-functioning phloem zone. (× 80). (1) Phloem ray cells; (2) necrotic sieve tubes and companion cells.



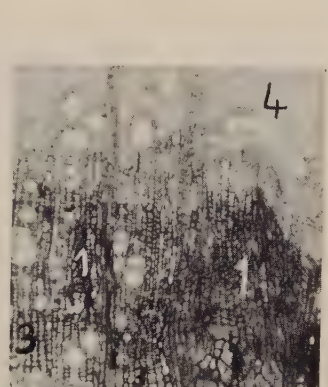
1



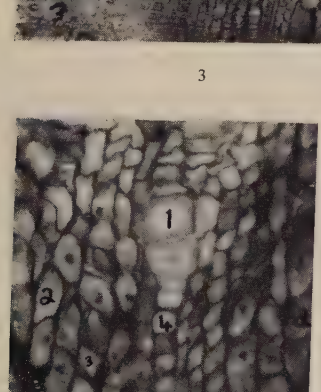
2



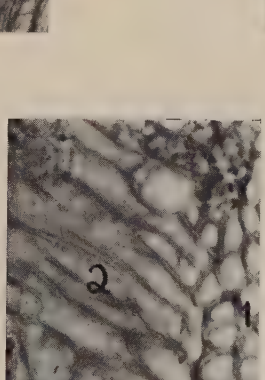
3



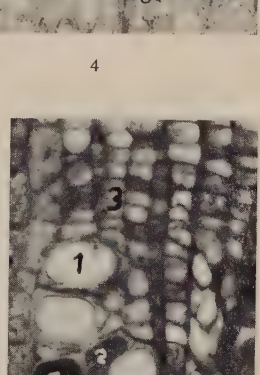
4



5

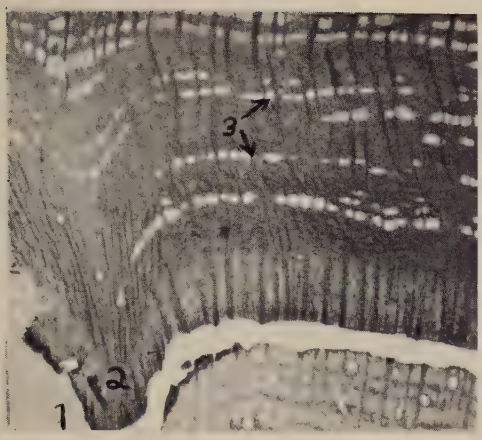


6

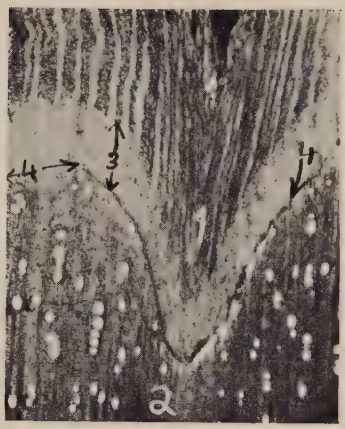


7

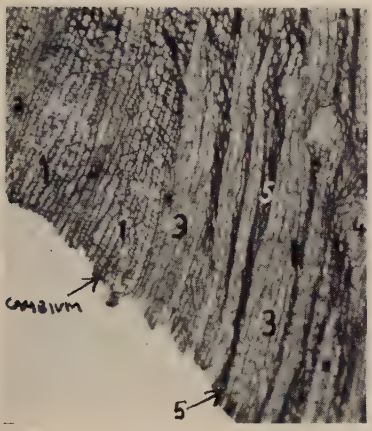
Plate III. Diseased wood. 1. Cross section showing inner wood lesions and distorted xylem tissue. (× 25). (1) Inner wood lesions; (2) kinked band of terminal parenchyma cells; (3) cambium; (4) small pits at cambium; (5) distorted xylem tissue. 2. Cross section of distorted xylem tissue shown in Figure 1. (× 80). (1) Abnormally orientated wood fibres; (2) abnormally orientated conducting vessels; (3) normal wood fibres; (5) normal medullary rays. 3. Radial section of distorted xylem tissue showing abnormal conducting vessels and wood fibres. (× 80). (1) Abnormal conducting vessels; (2) abnormal wood fibres; (3) medullary rays. 4. Cross section showing inner wood lesion. Cambium towards top. (× 80). (1) Lesion; (2) wound-parenchyma-like cells; (3) terminal parenchyma cells; (4) abnormally orientated conducting vessel and wood fibres. 5. Cross section of inner wood lesion. (× 400). (1) Abnormally small conducting vessel; (2) wound-parenchyma-like cells; (3) crystal idioblasts; (4) abnormal terminal parenchyma cells. 6. Cross section showing distorted wood fibres. (× 400). (1) Normal fibres; (2) tangentially distorted fibres. 7. Cross section showing gum-plugging of conducting vessel lumina. (× 400). (1) Normal conducting vessel; (2) gum-plugged vessel; (3) abnormal terminal parenchyma cells.



1

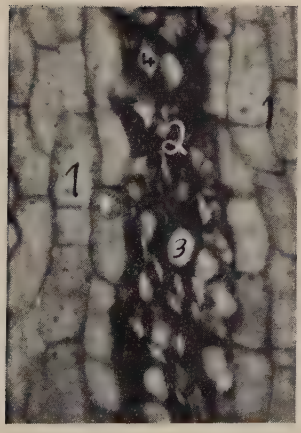


2



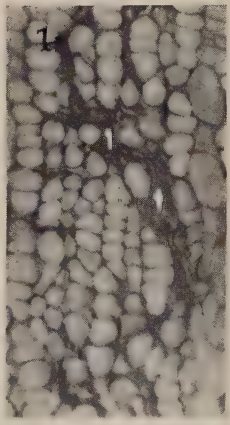
3

4



5

6



7

Plate IV. Diseased bark. 1. Cross section showing tendency of phloem fibres bundles to deflect into peg deformation. (× 25). (1) Xylem tissues; (2) peg deformation; (3) fibre bundles. 2. Cross section showing phloem peg wedged into wood. (× 25). (1) Peg deformation; (2) xylem tissues; (3) functioning phloem zone; (4) cambium. 3. Cross section of functioning phloem zone at side of peg deformation. Note extensive sieve tube necrosis. (× 80). (1) Normal sieve tubes; (2) normal phloem rays; (3) hyperplastic and hypertrophied phloem rays; (4) phloem fibre bundles; (5) necrotic sieve tubes. 4. Cross section of diseased bark of young rootstock showing outer bark abnormality. (× 25). (1) Peg deformation; (2) fibre bundles; (3) periderm; (4) triangular cavity appearing in centre of outer bark depression. 5. Cross section showing sieve tube necrosis inside peg tissues. (× 400). (1) Phloem ray cells; (2) necrotic sieve tubes; (3) parenchyma cell; (4) crystal idioblasts. 6. Cross section showing abnormal tissue in centre of peg deformation. (× 80). (1) Necrotic sieve tubes; (2) hyperplastic and hypertrophied phloem rays; (3) phloem fibres; (4) hypertrophied parenchyma cells. 7. Cross section showing sieve tube necrosis in part of functioning phloem zone where no peg deformations occur. (× 400). (1) Necrotic sieve tubes.

ROOTSTOCK—SCION INFLUENCES IN THE MORPHOLOGY AND ANATOMY OF THE BUD UNION OF SHAMOUTI ORANGE

SHULAMIT GOLDSCHMIDT-BLUMENTAL

Faculty of Agriculture of the Hebrew University, Rehovot

ABSTRACT

(1) A description is given of the morphology and anatomical structure of bud union of Shamouti with nine different rootstocks.

(2) The grafted rootstocks differ in their anatomical structure; especially in number and width of vessel clusters, amount of parenchymatic tissue, structure of rays, and number of idioblasts.

(3) Shamouti scions budded to various rootstocks display differences in the following anatomical characters: (a) number and cross section area of the vessels; (b) amount of parenchymatic tissue; (c) size of the rays; (d) number of idioblasts.

(4) The relative water conductive cross section of the Shamouti scions appears to be markedly affected by that of the rootstocks.

(5) The combinations of Shamouti scion with Shamouti, Baladi, sour orange, rough lemon and grapefruit stocks show a close correspondence between bud union morphology and the anatomic structure. In other combinations, morphological structure of the bud union and its anatomy do not show any correlation.

INTRODUCTION

In Israel, Shamouti orange is budded mostly to one of the two rootstocks, sweet lime and sour orange. On account of short-comings associated with both (Mendel 1936), a rootstock experiment was initiated in 1933 at the Agricultural Research Station, Rehovot. The experiment was designed to study rootstock influence on the Shamouti scion. Accounts of this work have been given by Oppenheimer (1936, 1940) and Mendel (1954).

In order to elucidate basic factors of compatibility between rootstock and scion, a study of the morphology and anatomy of the bud union of the Shamouti orange budded on various rootstocks was carried out in 1954. Whereas the studies of Mendel (1936) had been concerned with the anatomy of the actual union process, the present investigation was conducted on trees 20 years old. All nine rootstocks of the rootstock experiment were examined: (1) *Citrus limonia* Osb. (rough lemon), (2) *C. limonia* Osb. (sour lemon), (3) *C. aurantifolia* Sw. var. *dulcis* (sweet lime), (4) *C. medica* L. (citron), (5) *C. maxima* Merr. (shaddock Goliath), (6) *C. paradisi* Macf. (grapefruit Duncan), (7) *C. aurantium* L. (sour orange), (8) *C. sinensis* Osb. (sweet orange Baladi), (9) *C. sinensis* Osb. (sweet orange Shamouti).

Received April 15, 1956.

MORPHOLOGY OF THE BUD UNION

The morphological survey of the bud union was carried out in the groves of the Agricultural Research Station at Rehovot, the Agricultural School at Miqve Israel and the Citrus Demonstration Farm at Tserifin. The survey included 1060 trees on the principal rootstocks (560 on sweet lime, 420 on sour orange and 80 on rough lemon, as well as a smaller number of trees on the other rootstocks).

The following rootstocks were found to give a smooth union with the Shamouti orange: sour lemon, sour orange, Baladi orange, Shamouti orange and shaddock. In view of the small number of trees on shaddock included in the investigation, no general conclusions are warranted concerning the morphology of the bud union of Shamouti with this rootstock. Shamouti budded onto rough lemon exhibited the characteristic corky scar at the bud union, already described by Oppenheimer (1936, 1940) and Mendel (1954). This scar does not appear in other rootstock combinations with Shamouti. On citron rootstock (Figure 1), a medium to large swelling at the bud union was observed in all cases (Mendel 1954). Only one tree of the Shamouti—grapefruit combination survived, and even this tree was in very bad condition. Here the rootstock is much wider than the scion, a condition considered as typical for sweet oranges budded on grapefruit, according to Webber (1948) and recent observations by Mendel (verbal communication).

With Shamouti on sweet lime, the rootstock is generally wider than the scion (Figure 2); at the bud union, the trunk of the stock tapers gradually into that of the scion. This was observed in all the groves on light and medium soil. In heavier soils, the transition was more abrupt. It should be noted that trees of Shamouti on sweet lime suffering from xyloporosis show an overgrowth of the scion (Mendel 1954).

For the classification of the morphological types of bud unions of Shamouti with various rootstocks, we adopted the annotation system suggested by Webber (1948). A smooth bud union is indicated by *c* and, according to the degree of overgrowth of stock or scion, the signs + or — were added, respectively. Thus, the term '*c* +2' designates a bud union with strong overgrowth of the stock, like that found with grapefruit. The special morphological phenomena encountered in the present investigation with citron and sweet lime rootstocks, have led to the addition of two new ranks:

(1) The swelling of the bud union itself over the stock *and* the scion is given by the sign *D* (see Figure 1).

(2) The slow tapering of the rootstock trunk to that of the scion (Figure 2) is called type *E*.

Table I presents the results of the survey of bud union morphology for the main rootstocks.

THE ANATOMY OF THE BUD UNION

Materials and methods

Trees with typical bud unions were selected from the rootstock plantation. One bud union with a short part of the rootstock and scion was cut from every rootstock—

TABLE I

Bud union types of Shamouti orange with different rootstocks (percent of number of trees surveyed)

Rootstock	Type of bud-union				
	E	*c* + 1	*c*	*c* — 1	*D*
Rough lemon	—	14.9	70.5	14.6	—
Sweet lime	71.2	13.7	10.4	—	4.7
Sour orange	6.3	23.7	59.7	—	3.3

scion combination. The trunk pieces were then cut lengthwise, and the plane of the cut was orientated through the original position of the bud. One half of the trunk piece was smoothed and polished for macroscopic observations. For the anatomical investigations a slat 10—15 mm thick was cut from the second half. From every one of these slats, 6 blocks of wood (15 × 70 × 40 mm) were obtained; two each of rootstock and scion, 5—10 cm above and below the bud union respectively, and two from the bud union itself on opposite sides of the trunk.

As the unbudded rootstocks could not be cut off, the material for the investigation was obtained with the aid of hammer and chisel, but all the pieces splintered longitudinally.

All the wood samples were softened in a mixture of 1 part ethyl alcohol (95%) and 1 part glycerine. After five weeks, the material was cut in cross and tangential sections 15—20 μ thick. The sections were stained by the Pianeze BIII method. In this triple stain, malachite green stains lignified tissues greenish-blue, fuchsine turns the living tissues pink or red, while Martius' yellow imparts yellow or dark brown colour to cutinized tissues.

Unstained sections were used for the determination of the number and diameter of the wood vessels. Other anatomic tests were carried out on stained sections. Starch was determined by immersing the sections into a 5% J—JK solution.

Macroscopic observations

Macroscopic examination of the polished samples was made on the fresh material, immediately after cutting. A second examination was made one year later, after the pieces had dried up.

Whereas in the fresh material no striking differences in colour between the wood of the scion and the rootstock were generally discernible, the dried material showed in all combinations a deeper yellow colour of the rootstock as compared with the scion, except with Shamouti rootstocks, where no differences in colour between rootstock and scion were observed.

Necrotic islets were found in combinations of Shamouti with rough lemon, sour lemon and grapefruit. In the case of rough lemon these necrotic islets were in the same plane as the above mentioned corky scar, and appeared at every stage of tree development. In the combination with sour lemon, the islets are found rather close to the bark and are absent in the centre of the trunk. In grapefruit the necrotic areas are large and mostly constitute remnants of pruned shoots.

The anatomy of rootstocks and scions

Cross sections

The anatomical picture of the cross sections in general is as described by Mendel (1936) and Schneider (1952). The following should be added to their description:

(1) The differences in the number of vessels in the earlier and later layers of a growth ring and also the differences in width of these layers within a growth ring are characteristic of the various citrus species.

(2) There is no correlation between the diameter of the vessels and their location in the growth ring.

(3) In addition to the parenchyma layers observed by Mendel (1936), the clusters of vessels are also surrounded partially or completely by parenchymatic cells. The number of such parenchyma cells constitutes a characteristic feature of citrus species.

Tangential sections

In addition to the general anatomical features described by Mendel (1936) and Schneider (1952), it has been found that the height of the wood rays varies from three to twenty-four cells, and their width from one to five. The structure of the rays and the density of their distribution within the xylem are typical of the various citrus varieties examined.

Rows of large idioblasts (Esau 1953), furnished with lignified walls and containing one crystal of calcium oxalate (Schneider 1952), were found to accompany the rays in the lignified areas only. The rows contain from three (rarely one or two) up to twenty cells.

The main anatomic differences between the rootstocks and the scions were found in the number of vessels, in the cross-section area of the vessels and in the amount of parenchyma in the xylem.

The following table shows the number of vessels per mm^2 in a cross section of the trunks of the different rootstocks and their respective Shamouti scions.

TABLE II

Number of vessels per mm^2 in various rootstocks and their Shamouti scions (average of 50 counts)

Rootstock	Number of vessels in growth ring of rootstock		Number of vessels in growth ring of scion	
	Early production	Late production	Early production	Late production
Rough lemon	60.0	60.0	52.7	33.2
Sour lemon	46.3	46.3	45.0	30.8
Sweet lime	52.3	35.2	47.8	28.2
Citron	37.5	26.8	46.6	31.2
Shaddock	40.9	27.9	59.4	39.6
Grapefruit	34.1	19.8	32.8	19.5
Sour orange	32.4	20.5	31.8	25.2
Baladi orange	55.0	47.2	32.6	23.1
Shamouti orange	46.5	35.6	41.7	35.7

The table shows that the number of vessels per mm^2 is large in both the lemon-type and the orange-type rootstocks. The citron and the sour orange have a smaller number of vessels per unit area, and in this respect they resemble the grapefruit and the shaddock. It is possible that the degree of adaptation of rootstocks to the soil is reflected in the number of vessels. This might account for the fact that the rootstocks not suited to the soil of the experimental grove — such as sour orange, grapefruit and shaddock — have a smaller number of vessels per unit area. The problem undoubtedly calls for more thorough investigation.

The number of vessels in the rootstocks is larger than in the scions in all instances except for the Shamouti—shaddock combination. The number of vessels in the scion is obviously affected by the number in the rootstock. The correlations between number of vessels in rootstocks and that in scions were found to be significant. The respective correlation coefficients were:

(a) for early period in growth ring: $r = 0.874$ (highly significant)

(b) for late period in growth ring: $r = 0.693$ (significant at $P = 0.02$).

The cross section area of the vessels shows differences among the various rootstocks as well as variations in the Shamouti scions. Table III summarizes the vessel (single and double) areas in rootstocks and scion.

TABLE III

The cross-section area of the vessels in μ^2 (average of 50 measurements)

Rootstock	Rootstock		Scion	
	single vessel	double vessel	single vessel	double vessel
Rough lemon	3220	6470	4070	6680
Sour lemon	4430	9480	4930	8810
Sweet lime	2950	6780	3300	6100
Citron	5530	8790	5150	7910
Shaddock	1380	2370	4530	7910
Grapefruit	1820	3170	3300	6780
Sour orange	3220	8790	4530	8700
Baladi orange	5320	6780	4530	8700
Shamouti orange	2830	8290	3300	5820

* The cross section area of a vessel was calculated as an ellipse with the long and short diameter of the vessel as its axes.

It follows from Table III that in all combinations (except with citron, where the rootstock vessels are slightly wider) the vessels in the scion are wider than in the rootstock.

The area of the individual vessels in the scions fluctuates between $3300\mu^2$ and $5150\mu^2$. In rootstocks the variation is much greater: from 1380 to 5530 μ^2.

No correlations exist between the size of the cross section area of the vessels in the rootstock and that in the scion. There is also no evidence of any correlation between the number of vessels per mm^2 and their cross section area.

The amount of parenchyma in the xylem of the rootstocks corresponds closely to that in the unbudded seedlings. Again, the amount of parenchyma in the Shamouti scions shows correlation with that of their rootstocks (Table IV). There are two exceptions to this rule: (1) In rough lemon seedlings, the amount of parenchyma is small in the rootstock and large in the scion. (2) With citron, where the scion is well supplied with parenchyma while the rootstock has a low parenchyma content. Seedlings of citron have not been examined. This close correspondence between the amount of parenchyma in rootstock and scion (correlation coefficient $r = 0.674$, highly significant) indicates a strong influence of the rootstock in respect of this phenomenon.

TABLE IV

The amount of parenchyma in the xylem

(0 — *no parenchyma present*, 5 — *large amount of parenchyma*)

Rootstock	Tangential section			Cross section	
	Rootstock seedling	Budded rootstock	Shamouti scion	Budded rootstock	Shamouti scion
Rough lemon	1—2	4—5	3	4—5	3—4
Sour lemon	3	1	1	1—2	2
Sweet lime	5	3—4	3	2—3	4
Citron	6	1—2	4	5	3
Shaddock	4	4	3—4	4	4—5
Grapefruit	3	1	2	1	3
Sour orange	4	2	2—3	1—2	2—3
Baladi orange	4	2	2—3	3	3
Shamouti orange	3—4	1	0	1—2	0—1

The bud union

General description

The bud unions have been examined in tangential sections only. The exact location of the transition from rootstock to scion is not always identifiable. The bud union appears in all rootstocks as a more or less horizontal surface of irregular shape showing projections and indentations between the rootstock and scion tissues. Close to the bud union, remnants of rootstock tissues may be enclosed in the tissues of the scion and *vice versa*.

In undisturbed transition areas from rootstock to scion the anatomical picture resembles that of a normal citrus tree; vessels and fibres continue without any deviation from their vertical direction. The rays are straight and unbent. In some cases, however, a complex of twisted and intertwined tissues of abnormal structure can be observed. The fibres, vessels and pith rays are arranged around centres of variable size formed by groups of small, densely packed parenchyma cells. In some cases, the cells in these centres are necrotic. In stock—scion combinations with disturbed transition areas, starch accumulates in the living cells of the scion tissue. Jensen and his co-workers

(1927) connected this starch accumulation with the bud union morphology. In bud unions of Lisbon lemon on sour orange, the scion protrudes markedly over the stock, and the amount of starch found in the lemon tissue is higher than in the Lisbon—grapefruit combination, where the bud union is more or less smooth.

The bud union of Shamouti orange with various rootstocks

In the following description, we shall proceed from smooth, undisturbed combinations to the most irregular and complicated ones encountered in the course of our investigation.

In the bud union of Shamouti with Shamouti, Baladi and sour orange the transition from rootstock to scion has a normal appearance. No abnormal phenomena appear in the tissues of the union. The number of parenchyma cells at the transition point is very small. In the combination of Shamouti on Shamouti it is almost impossible to locate the exact line of transition. With Baladi the difference in the amount of idioblasts marks the transition point. In the Shamouti scion the number of idioblasts is average; in the rootstock they are almost absent. In the combination with sour orange, the transition is indicated by differences in the structure of the rays; in the Shamouti they are short and broad, and in the sour orange rootstock long and narrow.

No accumulation of starch was found in the scion tissue when Shamouti was budded to Shamouti seedlings. With Baladi stock the accumulation of starch is small, and with sour orange slightly larger.

In the bud union of Shamouti with sweet lime there is a deviation of all the vessels from the vertical direction. Small areas of necrotic tissue occur. Iodine staining shows a stronger accumulation of starch in the scion tissue than in the Shamouti—sour orange combination. This accumulation is most pronounced in the cells of the scion near the transition line. Starch grains are also found in the cells of the rootstock tissue, but fewer and more evenly distributed than in the scion.

In the bud of Shamouti with citron, slight contortions, which appear in the tissue near the transition, are limited to the scion only. The tissue of the rootstock is not disturbed. There are also obvious differences in the structure of the rays; they are wider in the scion than in the rootstock. Iodine staining shows average accumulation of starch in the scion tissue.

The bud union of Shamouti on shaddock presents major disturbances of the tissues, with strong contortions of stock and scion tissues. Moreover, areas may be found where the tissues are only slightly disturbed or even have a normal appearance. The position of the transition from rootstock to scion can easily be located because of the difference in the form of the rays: in the scion they are wider and higher than in the rootstock. Iodine staining shows large accumulations of starch in the scion.

The bud union of Shamouti and sour lemon is macroscopically clearly visible. The contortions of the tissues of stock and scion are very strong and evenly distributed. The number of parenchyma cells in the transition area is very large (Figure 3); many large centres of necrotic cells appear close to the bark. The rays are bent at the bud union.

They are wider and lower in the scion than in the rootstock. Iodine staining shows medium accumulation of starch in the scion tissue.

A similar structure can be seen in the bud union of Shamouti and rough lemon. The contortions in the tissues are still more pronounced and large necrotic areas are found, both in the bark and through the whole width of the wood. In addition, scion tissue is frequently included in rootstock tissue and *vice versa*. Iodine staining shows very strong accumulation of starch in the scion, which results in the formation of a black band above the bud union.

In the Shamouti—grapefruit combination, a broad area of twisted and contorted tissue precludes the determination of the exact boundary between rootstock and scion tissues (Figure 4). In all the combinations reported up to now, the disturbances of the tissues occurred within their normal planes of growth, but in the case of Shamouti on grapefruit the directions of growth become obliterated. A tangential section in the bud-union area may appear like a radial or even like a cross section. Very large necrotic areas are also present. Iodine staining shows considerable starch accumulation, comparable in extent to that occurring in Shamouti on rough lemon.

The water conducting capacity of the various stock—scion combinations

Differences in number and area of the vessels in the rootstock and scion undoubtedly affect the capacity for water and mineral transport from rootstock to scion. Data on the relative areas of the vessels in the trunks of rootstocks and scion are therefore of particular interest. The calculations are based on the data in Tables II and III, and the results are presented in Table IV.

TABLE IV

Relative areas of vessels in rootstocks budded with Shamouti (in percent of the cross section area of the trunk)

Rootstock variety	Relative vessel area	
	Rootstock	Scion (Shamouti)
Rough lemon	37.8	22.2
Sour lemon	34.7	25.1
Sweet lime	26.9	16.9
Citron	22.5	20.1
Shaddock	6.4	29.1
Grapefruit	7.7	13.5
Sour orange	20.2	19.2
Baladi	26.9	21.0
Shamouti	26.5	18.0

The table shows, that the relative water conducting cross section is bigger in the rootstocks than in the scions, except with grapefruit and shaddock, where the relation is reversed.

Figure 5 shows the close correspondence of the water conducting cross section between rootstocks and scions, with the exception of the shaddock rootstock, where apparently abnormal conditions prevail. Excluding the shaddock from the calculation. we arrive at a highly significant correlation between the relative vessel areas of the rootstock and the scion, the correlation coefficient being $r = 0.879$.

This correlation indicates a strong influence of the rootstock on the anatomical structure of the scion.

There appears to be a connection also between the relative water conducting cross section of the rootstocks and the vegetative development of the Shamouti trees budded to them. Basing the calculations on tree volume measurements by Mendel (1954), we have found a significant correlation between the corresponding values ($r = 0.653$).

DISCUSSION

Mendel (1954) describes the morphology of the bud union of Shamouti with various rootstocks. Our findings differ from his only in the combination with the sweet lime. According to Mendel (1954) the bud union is smooth in general, and only in some cases trees are found where the rootstock is wider than the scion. The results of our survey, which included a large number of trees, show that in most of the trees of Shamouti on sweet lime the rootstock is wider than the scion, and that the transition is not abrupt but marked by a gradual tapering of the rootstock trunk into that of the scion (Figure 1). This type of bud union was also found sometimes in combinations of Shamouti with other rootstocks; in those cases, the trees were showing symptoms of decline.

Another form of the bud union indicative of incompatibility, is a marked swelling of the union itself. Such swelling appears to be present in all trees budded to citron. The trees are medium sized to small. Mendel (1954) records that 56% of the trees on citron were attacked by diseases. Shamouti trees on other rootstocks, which show this swelling at the bud union, are declining and showing symptoms of disease.

Necrotic areas, macroscopically visible, were found at the bud union of Shamouti with grapefruit, rough and sour lemon. With grapefruit, these areas correspond to old pruning wounds associated with suckers arising near the bud union.

With rough lemon, the necrotic areas in the wood at the bud union correspond to the location of the corky scar described above (see also Mendel 1954, Oppenheimer 1940). These areas are found in the trunk from the earliest development stages of the budded tree. A specimen of a bud union of Shamouti budded on rough lemon in South Africa, and brought to Israel by Dr. Hanan Oppenheimer in 1936, exhibited the same symptoms. Oppenheimer also reported that, in South Africa, Shamouti does not succeed on rough lemon: the trees decline and die within three to four years after budding.

Recently, Childs et al. (1955) reported the occurrence of such corky scars in a number of Valencia orange trees budded to rough lemon.

With sour lemon, necrotic areas at the bud union are found only in the growth rings adjoining the bark. It thus appears that, while in the earlier years extensive

disturbances are confined to the tissues of the transition region (see above), conditions set in later which cause the death of the parenchymatic centres. The factors which are involved in bringing about these necroses are not known, but it is likely that they arise from cumulative physiological influences.

A pronounced effect of the rootstocks on the anatomical structure of the scion has been reported by other workers (see refs. 2, 3, 4, 10, 14, 15, 20, 21) in regard to trees of the Rosaceae family.

The effect of the rootstock on the anatomy of the scion finds expression in certain general indications:

(a) *Width and number of vessels.* We found that the width of the vessels of the scion is influenced by that of the rootstock. Colby (1935) reported an effect of the scion on the size of vessel clusters in apple rootstocks. Komarofsky (1947) found that, in addition to the effect of the rootstock on the size of the vessels of the scion, there is also an influence — though much weaker — in the opposite direction. Contrary to this, Beakbane and Renwick (1936) and also Herero (1951) did not detect any effect of the scion on the vessels of the rootstock. There is a close resemblance between our figures for vessel number in sour orange and sweet lime, and also their Shamouti scions, and the values reported by de Villiers (1939). Mendel's (1945) findings differ from ours, possibly due to the differences in plant material. Whereas Mendel worked with branches of young trees, in this work the trunks of mature budded trees were examined.

(*b*) *Water conducting capacity.* The compatibility of rootstock and scion appears to be conditioned to a considerable extent by their relative water conductive cross sections. It can be assumed that rootstocks with a relatively large vessel area would accelerate the growth of the scion. Conversely, a scion with a larger relative water conductive cross section than that of the rootstock, would be retarded in its growth.

In 20 year old trunks of sour orange and sweet lime budded with Shamouti, no appreciable differences in the water conductive cross section were found between the two species. In contrast to our results, Mendel (1945), working with young branches of the above species, reported that sweet lime had a much larger water conducting cross section than sour orange.

The structure of the bud union does not always indicate the degree of compatibility between rootstock and scion. In agreement with results of Bradford and Sitton (1929), we found that morphological structure of the bud union indicative of incompatibility is not necessarily associated with disturbed anatomical structure, and *vice versa.* As an example we might mention the union of Shamouti on citron: the bud union region is swollen, but the anatomy of this region does not show very marked deviations from the normal. On the other hand, the bud union of Shamouti on sour lemon is smooth, whereas the anatomical picture shows strong abnormalities.

Voechting (1892), in his grafting experiments with *Abies cephalonica*, found parenchymatic areas similar to those occurring in bud unions of citrus trees. Mosse (1953) described in detail the contortions of tissues at the bud union in ring grafts of apples.

She also found non-lignified parenchymatic areas which sometimes underwent subsequent lignification. Neither Voechting nor Mosse reported any occurrence of necroses in these areas. It should be borne in mind, however, that the trees examined by these workers were young, whereas our material was taken from adult trees.

Bradford and Sitton (1929) arrived at the conclusion that a union between rootstock and scion containing a large quantity of living tissue is not as strong as one which contains fibres. This would appear to be in line with our inference that a bud union furnished with a large amount of parenchyma indicates impaired compatibility, as compared with a bud union which contains a large amount of lignified elements and has tissues of a more normal appearance.

ACKNOWLEDGMENTS

The author wishes to express her thanks to Dr. K. Mendel, who suggested this study, for his valuable advice on various aspects of the investigation and for his assistance in the preparation of the manuscript. Thanks are due also to Dr. R. Volcani and Mr. J. Kalir for their help.

REFERENCES

1. Beakbane, A. B. and Renwick, M. E., 1936, A preliminary report on the internal structure of the wood of No. 9 rootstock in relation to scion-rooting of apples, *Rep. E. Malling Res. Sta.* 1935, 100—106.
2. Beakbane, A. B. and Thompson, E. C., 1939, Anatomical studies of stem and root of hardy fruit trees. The internal structure of the roots of some vigorous and some dwarfing apple rootstocks, and the correlation of structure with vigour, *J. Pomol.*, **17**, 141—149.
3. *Idem*, 1941, Anatomical studies of stem and root of hardy fruit trees. The anatomical structure of some clonal and seedling apple rootstocks, stem- and root-grafted with a scion variety, *ibid.*, **18**, 344—467.
4. Bradford, F. C. and Sitton, R., 1929, Defective graft union in the apple and pear, *Tech. Bull. Mich. agric. Exp. Sta.*, No. 99.
5. Colby, H. L., 1935, Stock—scion chemistry and fruiting relationships in apple trees, *Plant Physiol.*, **10**, 483—498 (quoted from Thompson).
6. Childs, F. L., Grant, T. S. and Grimm, S. R., 1955, A bud union abnormality of rough lemon rootstock with sweet orange scions, *Plant Dis. Reptr*, **39**, 810—811.
7. Esau, K., 1953, *Plant Anatomy*, Chapman and Hall, Ltd., London, 705 pp.
8. Herero, J., 1951, Studies of compatible and incompatible graft combinations with special references to hardy fruit trees, *J. hort. Sci.*, **26**, 186—237 (quoted from Thompson).
9. Jensen, C.A., Wilcox, L. V. and Foot, F. J., 1927, Behavior of different lemon bud and rootstock combinations in growth and yield at Limoneria, *Calif. Citrogr.*, **12**, 234, 266—268 (quoted from Webber 1948).
10. Komarofski, K., 1947, The wood anatomy of certain apple stock and scion varieties and correlative structural changes in the trunk induced by budding, *Palest. J. Bot. Rehovot*, **6**, 78—88.
11. Mendel, K., 1936, The anatomy and histology of the bud union in citrus, *Palest. J. Bot. hort. Sci.*, **1** (2), 13—46.
12. *Idem*, 1945, Orange leaf transpiration under orchard conditions, II, *Bull. agric. Exp. Sta., Rehovot*, No. 37, 59—85.
13. *Idem*, 1954, Stock—scion relations in a rootstock experiment with Shamouti on light soil, *Ktavim*, **5** (1), 5—30 (Hebrew).

14. Mosse, B., 1952, A study of the bark—wood relationship in apple stems, *Rep. E. Malling Sta* 1951.

15. *Idem*, 1953, The origin and structure of bridge tissues in ring grafted apple stems, *J. Pomol.*, 2 (1), 41—48.

16. Oppenheimer, H. R., 1936, A citrus rootstock trial on light soil, *Hadar*, **9**, 35—40.

17. *Idem*, 1940, The stock problem of the Shamouti orange, *ibid.*, **13**, 245—248.

18. Riker, A. J. and Riker, R. S., 1936, *Introduction to research on plant diseases*, John S. Swift Co Inc., St. Louis.

19. Schneider, H., 1952, The phloem of the sweet orange tree trunk and the seasonal production of xylem and phloem, *Hilgardia*, **12** (12).

20. Swarbrick, T. and Roberts, R. H., 1927, The relation of the scion variety to character of roo growth in apple trees, *Bull. Exp. Sta. E. Malling*, **78**, 1—23 (quoted from Thompson).

21. Thompson, E. C., 1952, Anatomical studies of stem and root of hardy fruit trees, *Rep. E. Malling Res. Sta.* 1951 (2).

22. De Villiers, E., 1939, The xylem anatomy, relative water conductivity and transport of dyes in citrus stems, *S. Afr. J. Sci.*, **36**, 291—313 (quoted from Mendel 1936).

23. Voechting, H., 1892, *Ueber Transplantation am Pflanzenkoerper*, Tuebingen.

24. Webber, H. J., 1948, *The Citrus Industry*, Vol. 2, pp. 69—168, University of California Press.

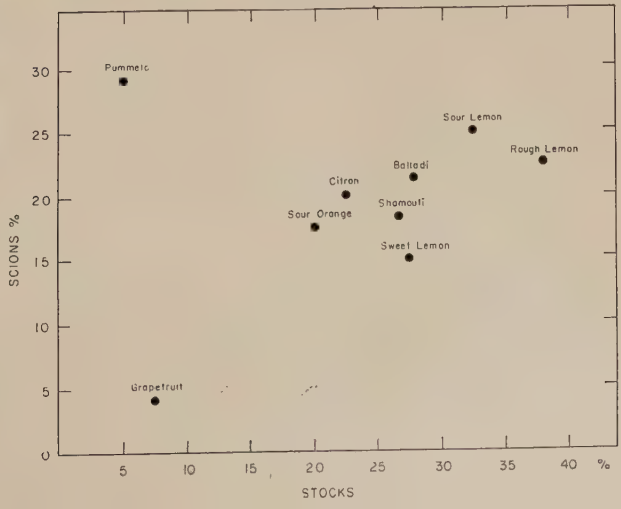
25. Webber, I. E. and Fawcett, H. S., 1935, Comparative histology of healthy and psorosis-affected tissues of *Citrus sinensis*, *Hilgardia*, **9** (2).

ıre 1. Bud union of Shamouti orange on sweet lime. Note the ıual tapering of the roots tock trunk into that of the scion.

Figure 2. Bud union of Shamouti orange on citron. Note the swelling of the bud union area.

igure 3. Bud union of Shamouti on sour lemon; tangential section. showing large centre of paren chyma cells. (×200).

Figure 4. Bud union of Shamouti on grapefruit: tangential sectio n showing contortions of xylem elements around small parenchymatic centre. (×109).



igure 5. Relative area of vessels in rootstocks and heir Shamouti scions (in percent of cross section of runk). Sweet lemon is synonymous with sweet lime.

ORANGE LEAF TRANSPIRATION UNDER ORCHARD CONDITIONS. IV. A CONTRIBUTION TO THE METHODOLOGY OF TRANSPIRATION MEASUREMENTS IN CITRUS LEAVES*

A. Halevy

Department of Horticulture, Faculty of Agriculture of the Hebrew University, Rehovot

ABSTRACT

Simultaneous measurements of transpiration have been carried out with the "Shamouti" orange tree using detached leaves of mature trees in a grove as well as two-year-old trees planted in tins. The hourly rates of transpiration and its daily march were compared. The experiments were conducted in normal autumn and summer, as well as on hot and dry *sharav* days. A striking resemblance in the shape of the daily transpiration curves was established under all conditions. On autumn days there was also great resemblance in the rates of transpiration in both types of weighings. On ordinary summer days the transpiration of the potted plants was lower by 25—35% than of detached leaves of the grove trees, and on *sharav* days it was as much as some 50% lower.

Successive weighing of detached leaves at one minute intervals disclosed that on autumn days there was no clear trend in the fluctuations of water loss till the 4th minute after plucking; later on there was evidence of an obvious trend towards decreasing rates, accompanied by stomatal closure. Conversely, on *sharav* and hot mid-summer days a sharp rise in transpiration was common. It set in during the 2nd minute after plucking and was accompanied by an opening of the stomata which had originally been more or less closed. Therefore, when measuring the transpiration of citrus leaves on summer days, it is essential to accomplish the initial and final weighings within 100 seconds after plucking. Under such conditions it is impracticable to return the leaves to their original site between the two weighings, as considered necessary by Konis (1950) for securing correct results. A hypothesis is advanced to explain the surprising opening of the stomata.

INTRODUCTION

The usual method of determining the transpiration rates of trees under field conditions consists of rapid weighing of detached leaves by means of a suitable balance, such as the torsion balance introduced by Huber (1927). Some authors (Konis 1950, Weinmann and LeRoux 1946) contest the reliability of the transpiration rates established by this method which, in their opinion, bears no fixed relation to the transpiration of intact plants. Therefore, before starting the studies on the transpiration of citrus leaves, tests had to be made as to the suitability of the method for the object of these studies.

---

* Abridged summary of Part I of a M. Sc. thesis presented to the Faculty of Agriculture of the Hebrew University in 1954.

Received February 19, 1956.

EXPERIMENTAL PROCEDURE

The above method is based on the supposition that, during the first minutes after detachment, the leaf continues to give off water vapour at the same rate as before separation from the plant. Konis (1950) showed that the usual way of exposing the leaves between the first and the second weighing on the hook of the balance, introduces considerable errors. In order to avoid these errors as far as possible, he suggests bringing the leaf back to its original position after the first weighing, exposing it at the original angle to the sun rays. This improved method, since used by various authors in Israel, is undoubtedly very valuable but is time-consuming. The question then arises, for how long after the plucking of the leaf does its physiological condition remain unchanged? In order to settle this question, losses in weight were measured during successive minutes, using 37 leaves, on 5.XI.1953. On the next day we examined the behaviour of the stomata under the same conditions by the infiltration method, using the scale of appraisement of their aperture proposed by Oppenheimer and Elze (1941).

The results of some of these weighings are shown graphically in Figure 1. We see that, till the 4th minute after detachment, the figures fluctuated without a pronounced trend. The differences were usually not larger than 25% of the first figure. They were small in the morning (0—13%), relatively large at noon (10—30%) and moderate in the afternoon (7—22%). But from the 4th minute on, the transpiration rates began to decline. The transpiration losses of the 5th minute were significantly lower than those of the 4th minute; losses during the 7th minute were still lower, and the difference was found highly significant. All the work was done with mature leaves and the results just cited relate to sun leaves only. In shade leaves the decline in transpiration set in a little later, i.e. in the 5th—6th minute. Actually the weighings were not always carried out at intervals of exactly 60, but rather 50—75 seconds. The rates were calculated in milligrams water loss for gram leaf weight per hour.

As to the stomata, there were no very obvious changes before the 7th minute after detachment of the leaves. As a rule, closing movements set in between the 7th and the 10th minute. The closure continued slowly, and the stomata closed definitely about 30—40 minutes after plucking. Leaves whose stomata were completely closed when plucked, invariably remained closed afterwards.

In order to minimize injury to the mesophyll by the infiltration liquids (as a rule, kerosene), small drops only were applied and the unabsorbed kerosene was soaked up with blotting-paper after 20 seconds. Both days on which the tests were made were typical autumn days. The temperature was not excessively high (20—29°C) and the air was not excessively dry (50—85%). It can be concluded that on such days no great changes in the transpiration took place in the first four minutes after plucking. Therefore it seems feasible to work under these conditions according to the recommendations of Konis.

However, when examining Shamouti leaves in the hottest hours and especially on *sharav* days (very dry and hot days in spring and autumn), a quite different and rather surprising behaviour was found. When the leaves, whose stomata were closed as a

reaction to the great water deficit of the atmosphere, were examined, it was found that the stomata opened shortly after the detachment of the leaves. 225 leaves taken from 5 trees were examined during three *sharav* days (April 16, May 24, June 6, 1953). On the first day the plantation was awaiting its first irrigation. The results were: in 83% of the mature leaves and 86% of the young, light-green leaves, the stomata opened after plucking. Of each of these groups, if the total of leaves with opening stomata is 100%,

Mature leaves	Young leaves	
6%	11%	opened before the end of the 2nd minute
32%	41%	opened in the 3rd minute
38%	36%	opened in the 4th minute
24%	12%	opened after the 4th minute

The stomata did not remain open, but began to close again usually 10—15 minutes after the plucking, arriving at a state of complete closure soon afterwards.

It was found that this behaviour occurs not only under the extreme conditions of *sharav* weather but also at mid-day on an ordinary summer day (temperature 29.5°C, R.H. 54%) four days after irrigation. Of the leaves examined on such days 57% opened their stomata; more than one half had already opened before the end of the 3rd minute.

On another *sharav* day (6.VI.1953) a series of successive weighings were again taken, using leaves with closed stomata. Of the 29 leaves examined, in 22 a sudden increase of the transpiration soon after plucking was found.

The march of transpiration of some of these leaves is shown in Figure 2. It shows that with the opening of the stomata a large increase in transpiration occurs which amounts to thrice and more the rate of transpiration in the first minute. It can be concluded that on *sharav* days and around noon on summer days an effort should be made to finish the first weighing not later than 20 seconds, and the second one, not later than 80—100 seconds after severing the examined leaves. Shortage of time does not allow the leaves to be brought back to their former position on the tree. It was, therefore, found satisfactory to leave the shade leaves on the balance (which, of course, was placed in the shade) between the two weighings, leaving the cover-box, in which the leaves are placed during weighing, wide open. Sun leaves are removed from the hook very quickly and hung in the nearest sunny place. It is believed that on such days the speed of the measurement is more important than exposing the leaves in the same position they had occupied previously on the tree.

In order to study the relationship between transpiration rates of detached leaves and those on the tree, simultaneous weighings were undertaken, of detached leaves in a grove and of three intact young trees in 25 litre tins. The leaves were of Shamouti orange trees, budded on sour orange stock. The trees in the grove from which the detached leaves were plucked were about 20 years old. The trees in the tins were two years old. The tins stood on a southern balcony in the Division of Citriculture of the Agricultural Research Station about 30 metres from and about 8 metres higher than

the trees. Every day, before the first weighing, the tins were wrapped in a plastic envelope impenetrable to water vapour. At the end of each day's measurement the contours of all the leaves of the balcony trees were traced on paper and their area was measured planimetrically. The same was done with detached leaves after each measurement. The following curves show the average transpiration as losses in weight of the tree tins for every hour. The curves of the detached leaves show the average transpiration rates for every half hour. As the tins usually stood on the sunny terrace and since their trees were young and their foliage sparse, we may say that all the leaves were practically sun leaves. Therefore, when comparing their transpiration with that of mature trees in the grove, we chose for comparison of the latter only sun leaves from the southern side. In order to avoid the depressing influence of dry soil on transpiration (Furr and Degman 1932, Martin 1940, Schneider and Chillers 1941, Mendel 1954, 1951, Brueckner 1945, Loustalot 1945), the tins were irrigated every morning preceding the days when measurements were carried out. The former simultaneous weighings were carried out on 7 days: 3 summer days, 2 *sharav* days, 2 autumn days. The transpiration curves of the days in every climatic group are similar, so we cite here only one curve of each type. In addition to the transpiration curves, the curves of meteorological data are shown; evaporation (Piche evaporimeter), temperature and humidity (psychrometer) and the condition of the stomata as established by the infiltration method with kerosene, according to Oppenheimer and Elze (1941).

On the autumn days (Figure 3) we found excellent correspondence of the results established on different objects with different weighing methods. Not only were the daily march of the transpiration and the shape of the curves similar, but also the rates of transpiration were about the same. It would seem that the resemblance is due to the moderate weather conditions: low temperature, high humidity and a cloudy sky during almost the whole day. All these produced more or less the same average microclimatic conditions and only average deficits in the deep-rooted trees in the grove and in the trees in tins on the balcony. Therefore similar transpiration was found in response to these conditions.

On summer days (Figure 4), the shape of the curves was also similar to that in Figure 3, but the transpiration rates in the potted trees were lower throughout the day. But while the differences were small in the morning and in the afternoon, they were high during the mid-day hours. The resemblance of the rates in the morning and in the afternoon — when the temperature, the saturation deficit of the air and the radiation of the sky were low — confirms the opinion arrived at regarding transpiration on autumn days. The lower transpiration of trees in containers can be explained by two main assumptions:

(a) The young trees in the containers (although painted white), having had their water expense restricted, suffered from an inner water deficit especially during the hottest hours of the day, as a consequence of strong heating of the containers and the soil. This effect was probably increased by reflected radiation from the white walls of the building behind the containers.

(b) Their condition caused by this water deficit was probably aggravated by the restriction imposed on the development of the root system, and injury to peripheric roots reaching the heated walls of the container.

The results of the measurements on *sharav* days are shown in Figure 5. Here again the daily march of transpiration of the potted nursery trees resembled that of the mature trees. But the differences in the rates (height reached by the graphs) are even greater. In one case the first peak before noon reached 678 mg/dm²/h in the former as against 1669 in the latter. In the afternoon 516 mg/dm²/h was found in the tinned saplings, but 1134 in the leaves of the mature trees (in the grove). It may be assumed that the factors responsible for this relative reduction of water expense on typical summer days were much more effective on *sharav* days. The high water deficit of the saplings caused reduction in stomatal aperture. After a short opening period in the morning, the stomata were found closed between 8 and 9 o'clock, and remained so almost throughout the day. In contrast to this, the closure of the stomata of the mature trees set in later, and the stomata opened again in the afternoon.

In general the peaks in the curves of the saplings appear a little later than in the detached leaves. It is assumed that this is only a consequence of the method of graphical presentation. The points of the former curves symbolize the results of one weighing at the *end of every hour* which were plotted accordingly, while in the detached leaves the curves represent averages of successive weighings, carried out *every half hour*. But this of course did not change additive figures of daily transpiration, as can be seen from the following table:

TABLE I

Daily transpiration losses per unit of leaf surface of Shamouti saplings in containers and of detached leaves from mature trees

(mg/dm²/h sun leaves)

Date	Meteorological condition	Saplings in containers weighed *in toto*	Leaves of mature trees (torsion balance)	Relative transpiration of saplings (%) (detached leaves from mature trees = 100)
14/4/53	Summer day	8.112	11.136	73.1
25/6/53	Summer day	8.094	12.829	63.7
31/6/53	Summer day	7.973	12.048	64.6
6/5/53	Sharav day	4.045	9.105	44.5
14/5/53	Sharav day	4.978	10.216	48.7
3/11/53	Autumn day	5.436	5.621	96.5
9/11/53	Autumn day	6.043	6.664	90.5

The data of this table show only very slight variations on autumn days (4—10%) and moderate variations on summer days (not more than 35%). It is important to note that in all cases the water losses calculated per unit of leaf surface of the plants in tins were smaller than those of the detached leaves.

Summing up the experimental evidence, it was found that the good agreement of the shape of the curves in all the cases and of the rate of transpiration under moderate atmospheric conditions suggested that — in spite of certain obvious limitations — the use of the quick weighing method of detached leaves seems adequate for the study of water use by citrus plants naturally rooted in the soil.

DISCUSSION

The methods of transpiration measurement

In the previous chapter we mentioned Konis' (1950) opinion that the quick weighing method is unreliable. He found a lack of correspondence in the rates of transpiration even in leaves growing near each other on the same branch and he explained these large deviations by differences of leaf temperature produced by different exposure to the sun rays. This opinion is well founded, but Konis relies in his criticism upon measurements made in June-July, when the leaves were exposed for $2\frac{1}{2}$ minutes between the first weighing and the second, while another $\frac{1}{2}$ minute passed before the first weighing. According to our experience this is too long in the case of citrus leaves examined in the open during these months. Therefore it is possible that part of the unfavourable results of this investigation is due to the way it was carried out. Unfortunately no mention is made whether or not the aperture of the stomata was examined. Transpiration measurements of detached leaves in successive minutes after plucking were carried out by Oppenheimer and Mendel (1939) in citrus and recently by Ferri (1953) and Ferri and Labouriau (1952) in plants of the Brazilian Caatinga (dry forest) and by Pisek and Cartellieri (1942) in the Alps. Our results resemble those of Oppenheimer and Mendel. From Ferri's work we learn that there are leaves reducing their water loss to one half of their original figure already in the second minute after the detachment, while others undergo no change in their transpiration even after half an hour or more. Therefore one has to examine the behaviour of every plant species before starting the study of its transpiration by the rapid weighing method. Several authors compared transpiration of plants with that of detached leaves. Weinmann and Le Roux (1946) undertook investigations with barley, oats, maize and wheat; they found only little correlation. Consequently they concluded that "the determination of the water loss from the cut plant cannot be regarded as a reliable index of the true transpiration rate of the intact plant". Kaplan (unpublished) compared the two methods in investigating the transpiration of Eucalyptus. He found resemblance in the daily march of transpiration but got always lower rates in the potted plants. Evenari (1953) examined some desert plants. He found very similar rates in herbaceous plants, but in woody plants transpiration calculated from losses in weight of detached leaves was not equal to averages calculated from losses of entire plants, because in such plants every leaf is subject to different microclimatic conditions.

We do not know about work of this kind in citrus except the incomplete work of Elazari-Volcani (1938), who found a fair degree of agreement in the march of daily

transpiration curves, though not of the absolute figures when examining sour oranges by the two methods (cf. Oppenheimer and Mendel 1939).

In contrast to the findings of Evenari (1953) and Weinmann and Le Roux (1946) indicating that the transpiration of the intact plants was sometimes higher and sometimes lower than that of the detached leaves, we invariably found lower rates in the potted plants. This is in line with the results of Ivanov (1950) and Kaplan.

The opening of the stomata after detachment of the leaves

Regarding our unexpected observation that opening of the closed stomata and rising transpiration values occur after leaves have been detached on *sharav* days and on hot summer days, identical indications were not found in the literature on the subject, although similar phenomena have been reported for other plants and under different conditions.

Iwanoff (1928) in Leningrad found an increase in transpiration after severing branches from forest trees. He attributed it to the influence of the concussion suffered by the leaf at the moment of plucking, which shock, in his opinion, is responsible for a relaxation of stretched threads of water in the vessels. This, in turn, raises the vapour pressure in the intercellular spaces of the leaf and thus favours transpiration. Iwanoff does not remark on the condition of the stomata. Under the conditions of his study, the saturation deficit of the air was certainly rather low, therefore we do not consider his observations as analogous to our own findings. In a recent paper Ivanov et al. (1950) observed a sudden rise in transpiration immediately after cutting off leaves or leafy branchlets. The authors assume that this increase is associated with a sudden release of stress in the water columns. Here again we are not informed about the condition of the stomata, but this rise of transpiration occurs within the first minute after cutting. Therefore it seems that it is not identical with the phenomenon observed by us which does not set in before the second or third minute. Yet, we do not wish to exclude the possibility that in addition to the relatively late rise in transpiration caused by the opening of the stomata, there may have been in our case also an earlier increase immediately after severing of the leaves.

Bakke (1914, 1918) performed a series of experiments on sunflower plants. He found that during progressive wilting there was a gradual drop in transpiration, followed by a period of stable conditions, and later on an abrupt rise in rate just beyond the permanent wilting condition.

Knight (1922), who worked with detached shoots, made simultaneous measurements of transpiration and of stomatal opening during progressive stages of wilting. He found a sudden increase in stomatal opening, accompanied by an increase in transpiration at an early stage of wilting, followed finally by a gradual drop. Thus, it can be seen that Knight (1922) found an increase in transpiration at the beginning of wilting and Bakke (1918) at its end.

Our findings are similar to those of Knight, in spite of the difference in climatic conditions. While Bakke and Knight worked under laboratory conditions in Europe

and did not meet great water deficits, we worked in Israel in an orchard under dry weather conditions.

Zalenski (1921), working in Central Asian deserts, found that during periods of hot and dry winds the stomata of some plants which had previously been closed (because of lack of water) — opened. This caused a rise in transpiration and ultimately drying up of the plants.

Franco and Inforzato (1950), having examined the transpiration of coffee plants, stated that stomata which originally had been half-open, opened widely after the examined leaves had been detached from the plants.

Mendel (1945, pp. 70, 77), who investigated orange leaves, found that on a certain summer day (6.X.1938) transpiration was unusually high and apparently not at all influenced by stomatal movements, but showed marked parallelism to the changes of meteorological factors. He explains this unusual behaviour by injury caused to the cuticle of the leaves, which rendered an increase in transpiration possible even when the stomata were closed. According to our own results, may we suggest an alternative explanation? Mendel's findings were possibly caused by wide opening of the stomata after detachment, which escaped his attention. Such opening of the tightly closed stomata was found by us, as mentioned before, even under ordinary summer conditions a few days after irrigation. Before we became aware of the opening of the stomata in the second or the third minute, we were also at a loss how to explain the lack of agreement between high transpiration figures and extremely low ones of original stomatal opening.

In the following lines we want to suggest an explanation of this phenomenon, in the light of the theory of water balance. On *sharav* days and midsummer days the stomata close as a consequence of a negative balance, according to Stalfelt's hydroactive mechanism (1932).

But the water loss continues through the cuticle and probably through submicroscopical slits of apparently closed stomata, because of the enormous suction force of the dry air. This, according to Crafts et al. (1949), amounts at 50% R.H. to 923 and at 10% — to not less than 3067 atmospheres (figures for 20°C). In an initial stage of adverse atmospheric conditions, the plant still maintains the equilibrium between transpiration and absorption, by closing the stomata and by internal adjustments. But later on, under extreme conditions of dryness and especially during dry winds, it is unable to keep up this balance, and the leaves wilt and die. Maximov (1929), Zalenski (1921) and others established this behaviour in other plants, and Reed and Bartholomew (1930) in citrus. We may assume that leaves whose stomata were closed because of the great moisture deficit of the air were in the initial stage of stress. When we plucked such leaves, the water loss continued at the previous rate or even increased.

This, according to a suggestion which we submit here for the first time, leads to a rupture of the water columns in the leaves, which had been under tension. The released water produces a sudden increase of turgidity in the guard cells; the stomata open and we notice a sharp rise in transpiration rates. Later on, after additional dwindling of

the leaf's water reserves, the turgor in the guard cells decreases again and then the stomata close finally.

Another possibility is the following: According to the hydro-passive mechanism of stomatal movement defined by Stalfelt (1932), abundance of water supply produces a high turgor pressure in the epidermis cells which leads to mechanical closure of the stomata. One can imagine that the opposite happens in our case. As a consequence of heavy water loss, the turgor pressure in the neighbouring epidermis cells possibly decreases to such an extent that they shrink, drawing the guard cells apart, and producing in this way a passive opening of the stomata.

ACKNOWLEDGMENT

The writer wishes to express his sincere thanks to Prof. H. R. Oppenheimer, who suggested this study and helped in the revision of the English summary. He is further obliged to Dr. S. P. Monselise for guidance and useful advice, and to Mr. A. Shavit and Mr. A. Bielorai, who helped in the execution of the experiments.

REFERENCES

1. Bakke, A. L., 1914, Studies on transpiration power as indicated by the method of standardized hygrometric paper, *J. Ecol.*, **2**, 143—173.
2. *Idem*, 1918, Determining of wilting, *Bot. Gaz.*, **66**, 81—116 (cited from Crafts et al. 1949).
3. Brueckner, A. E., 1944, Transpiration studies of some Natal midlands Thornveld trees, *S. Afr. J. Sci.*, **41**, 186—193.
4. Crafts, A. S., Currier, H. B. and Stocking, C. R., 1949, *Water in the physiology of plants*, Chronica Botanica, 420 pp.
5. Elazari-Volcani, 1938, T., *Do transpiration weighings of trees in containers render values approaching natural losses?* (unpublished manuscript).
6. Evenari, M., 1953, The water balance of plants in desert conditions, *Proc. Int. Symp. Desert Res.*, Jerusalem 1952, Research Council of Israel, 266—274.
7. Ferri, M. G., 1953, Balanço de água de plantas de caatinga, *Anais do IV Congr. Nac. Soc. Bot. Brasil*, 314—332.
8. Ferri, M. G. and Labouriau, G., 1952, Water balance of plants from the "caatinga". I. Transpiration of some of the most frequent species of the caatinga of Paulo Alfonsa (Brazil) in the rainy season, *Rev. bras. Biol.*, **12** (3), 301—312.
9. Furr, J. R. and Degman, E. S., 1932, Relation of moisture supply to stomatal behaviour of apple, *Proc. Amer. Soc. hort. Sci.*, **28**, 547—551.
10. Franco, C. M. and Inforzato, R., 1950, Quandidade de água transpirada pelo caféeiro cultivado ao sol, *Bragantia*, **10** (9), 247—257.
11. Huber, B., 1927, Zur Methodik der Transpirationsbestimmung am Standort, *Ber. dtsch. bot. Ges.*, **45**, 611—618.
12. Ivanov, L. A., Simila, A. A. and Tselniker, Y. L., 1950, On the rapid weighing method for determining transpiration under natural conditions (in Russian), *Bot. Zh. S.S.S.R.*, **35** (2), 171.
13. Iwanoff, L., 1928, Zur Methodik der Transpirationsbestimmung am Standort, *Ber. dtsch. bot. Ges.*, **46**, 306—310.
14. Kaplan, Y., *On the ecology of Eucalyptus rostrata*, unpublished thesis.
15. Knight, R. C., 1922, Further observations on the transpiration, stomata, leaf water content and wilting of plants, *Ann. Bot.*, **36**, 361—383 (cited from Crafts et al. 1949).

16. Konis, E., 1950, The effect of leaf temperature on transpiration, *Ecology*, **31** (1), 147—148.

17. Loustalot, A. J., 1945, Influence of soil moisture conditions on apparent photosynthesis and transpiration of pecan leaves, *J. agric. Res.*, **71** (12), 519—532.

18. Martin, E. V., 1940, Effect of soil moisture on growth and transpiration in *Helianthus annuus*, *Plant Physiol.*, **15**, 449—466.

19. Maximov, N. A., 1929, *The plant in relation to water* (English edition by Yapp), London, 451 pp.

20. Mendel, K., 1945, Orange leaf transpiration under orchard conditions. II. Soil moisture content decreasing, *Palest. J. Bot. Rehovot*, **5** (1), 59—87.

21. *Idem*, 1951, Orange leaf transpiration under orchard conditions. III. Prolonged soil drought and the influence of stocks, *ibid.*, **8** (1), 45—54.

22. Oppenheimer, H. R. and Mendel, K., 1939, Orange leaf transpiration under orchard conditions. I. Soil moisture high, *ibid.*, **2** (2), 171—250.

23. Oppenheimer, H. R. and Elze, D. L., 1941, Irrigation of citrus trees according to physiological indicators, *ibid.*, **4** (1), 20—46.

24. Pisek, A. and Cartellieri, E., 1942, Der Wasserverbrauch einiger Pflanzengesellschaften, *Jb. wiss. Bot.*, **90**, 255—291.

25. Reed, H. S. and Bartholomew, E. T., 1930, The effects of desiccating winds on citrus trees, University of California, Agricultural Experiment Station, Bulletin No. 484, 59 pp.

26. Schneider, G. W. and Childers, N. F., 1941, Influence of soil moisture on photosynthesis, respiration and transpiration of apple leaves, *Plant Physiol.*, **16**, 565—584.

27. Stalfelt, M. C., 1932, Der stomataere Regulator der pflanzlichen Transpiration, *Planta*, **17**, 22—85.

28. Weinmann, H. and Le Roux, M., 1946, A critical study of the torsion balance for measuring transpiration, *S. Afr. J. Sci.*, **42**, 147—153.

29. Zalenski, V., 1921, Ueber die physiologische Wirkung des Hoehenrauches auf die Pflanzen, *Bull. agric. Exp. Sta., Saratov*, **3** (1—2), 1—21 (cited from Maximov 1929, p. 384).

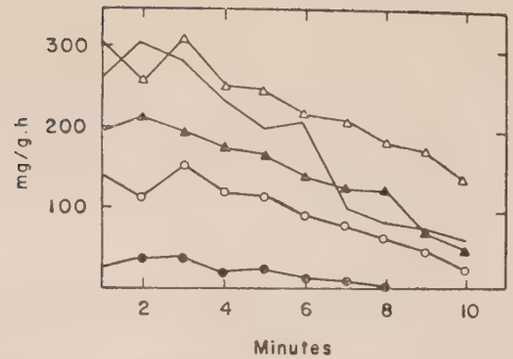


FIGURE 1. Transpiration of detached Shamouti leaves in the first 10 minutes. Autumn day.

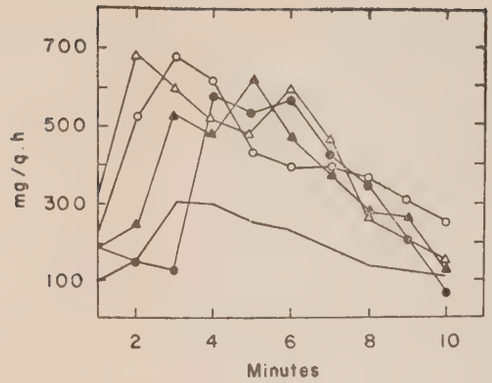


FIGURE 2. Transpiration of detached leaves in the first 10 minutes. Sharav day.

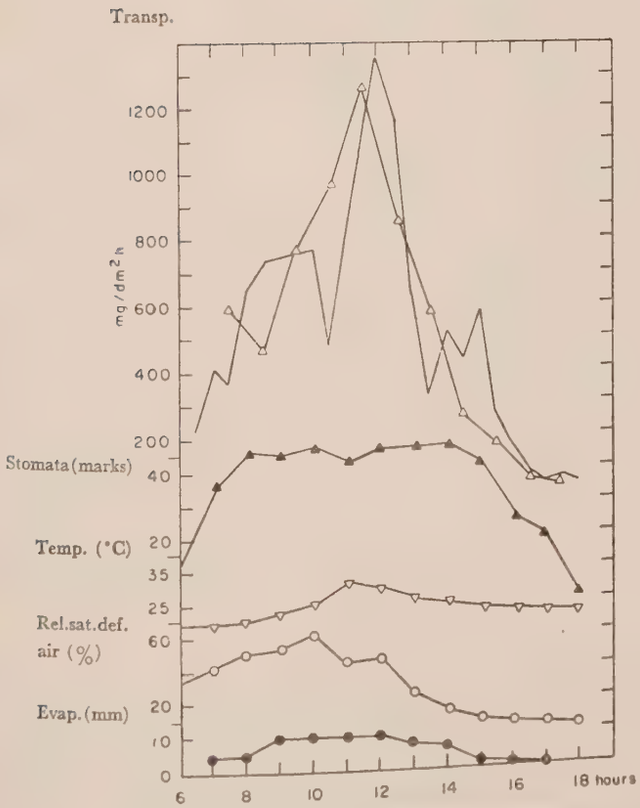


FIGURE 3. Simultaneous transpiration of detached Shamouti leaves plucked in the grove and non-detached leaves of potted saplings. Sun leaves. Autumn day (3.2.53).
Transpiration ———— of detached leaves
Δ——Δ of potted saplings

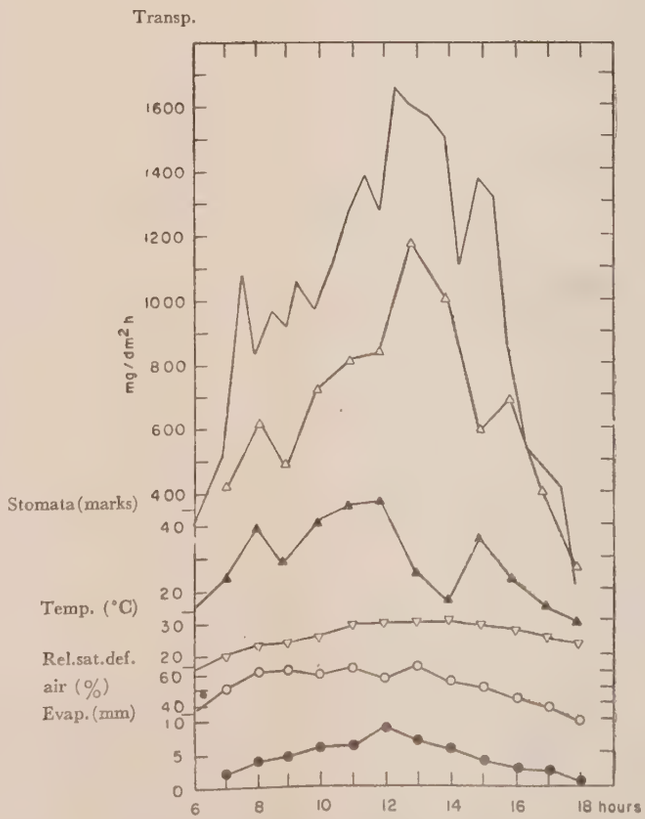


FIGURE 4. Simultaneous transpiration of detached Shamouti leaves plucked in the grove and non-detached leaves of potted saplings. Sun leaves. Normal summer day (31.5.53).
Transpiration ————of detached leaves
Δ——Δ of potted saplings

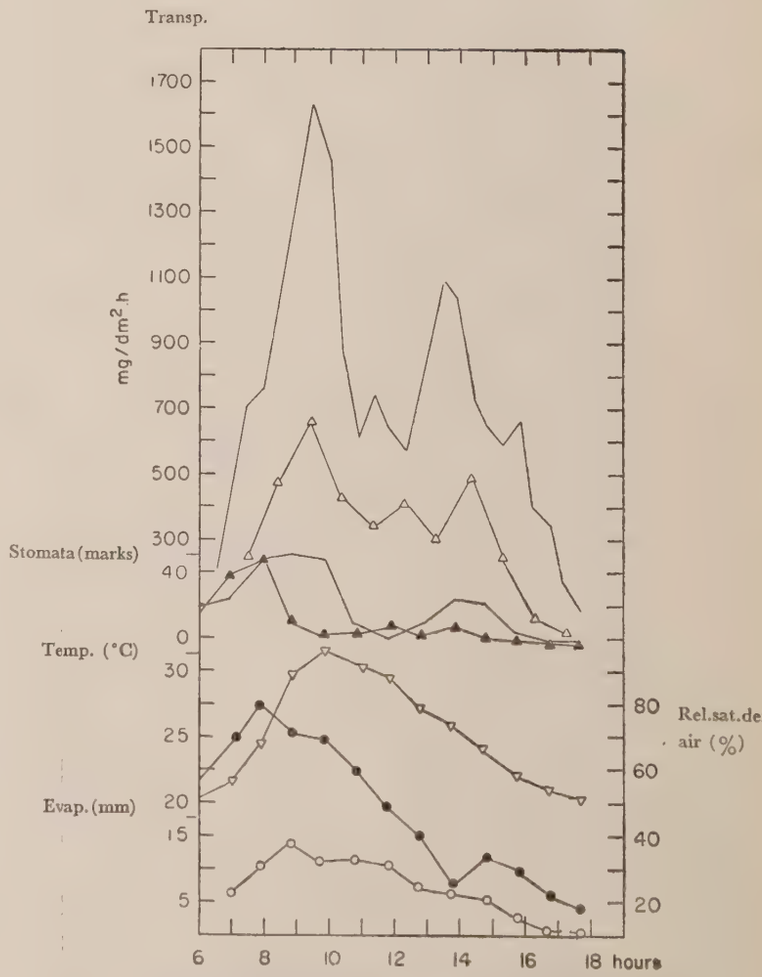


FIGURE 5. Simultaneous transpiration of detached Shamouti leaves plucked in the grove and non-detached leaves of potted saplings. Sun leaves Sharav day (6.5.53).
Transpiration ———— of detached leaves
Δ——Δ of potted saplings

ORANGE LEAF TRANSPIRATION UNDER ORCHARD CONDITIONS. V. INFLUENCE OF LEAF AGE AND CHANGING EXPOSURE TO LIGHT ON TRANSPIRATION, ON NORMAL AND DRY SUMMER DAYS

A. Halevy

Department of Horticulture, Faculty of Agriculture of the Hebrew University, Rehovot

ABSTRACT

(1) The main purpose of this work was to examine the transpiration of the Shamouti orange leaves on Sharav days compared with transpiration on normal summer days. Sharav or Khamsin is hot and dry air penetrating from continental areas bordering upon Israel, mainly from the east.

(2) The daily curve of transpiration on normal summer days showed usually two peaks, which were about the same height, but varied according to meteorological conditions. The curve had occasionally three peaks, of which the middle one was the highest. On Sharav days, the curve had two or three peaks; the first being always the highest, and occurring earlier than on normal days, especially so in the second or the third consecutive day of Sharav.

(3) On Sharav days, the daily transpiration losses per unit of leaf surface did not exceed those on ordinary summer days, and usually they were even smaller.

(4) The daily transpiration losses of shade leaves were always lower than those of sun leaves, but the differences were smaller on Sharav days than otherwise; the reason being that on such days the stomata of sun leaves close earlier in the forenoon, so that during the hottest hours the shade leaves transpire much more than the sun leaves.

(5) A direct correlation could mostly be established between transpiration rates and stomatal aperture. In some instances, however, a decrease of the transpiration rates was recorded without any considerable change in the condition of the stomata.

(6) Four types of leaves were distinguished according to their age:
- (a) Very young leaves which have the highest transpiration figures.
- (b) Young, light-coloured leaves that had attained their final size, with transpiration lower than that of mature leaves.
- (c) Mature leaves.
- (d) Old leaves, nearing the age of abscission, which showed the lowest transpiration losses.

(7) Both daily and short period ($1^1/_2$—2 minutes) transpiration losses were lower than those established by Oppenheimer and Mendel (1939).

INTRODUCTION

In an earlier paper Oppenheimer and Mendel (1939) studied orange leaf transpiration on single days in all months of the year. Among these were two Sharav days (19.3.1935 and 29.5.1935). They found that transpiration losses were not higher than those on a normal summer day. This surprising result aroused interest in further examinations

Received March 14, 1956.

and search of evidence, in view of the practical and theoretical importance of the problem Thus Prof. H.R. Oppenheimer suggested the present work on transpiration of orange leaves on Sharav days compared to the transpiration on ordinary summer days. In our investigations we distinguished between sun and shade leaves, as well as between mature, dark-green and young, light-green leaves. Special attention was paid to the distinction between the two age groups, considering the differences of opinion concerning their relative respiration rates (Oppenheimer and Mendel 1939, Boeuf and Genet 1906, Bartholomew 1931).

The experiments were carried out with 23 years old Shamouti orange trees, budded on sour orange, in a grove of the Agricultural Research Station at Rehovot. Detailed descriptions of the soil and the climatic conditions of Rehovot can be found in the earlier papers of Oppenheimer and Mendel (1939) and Mendel (1945, 1951).

On Sharav (or Khamsin) days the temperature is high, while the relative humidity is very low, sometimes less than 10%. These conditions produce very strong evaporation, at times as much as thrice the amount on normal summer days (Ashbel 1939). However, in contrast to similar hot-dry spells in other countries, the eastern, southern or southeastern winds blowing on Sharav days in Israel are usually very slow and the radiation of the sky is rather weak (Ashbel 1934-5).

METHODS

Transpiration losses were measured by weighing detached leaves on Huber's (1927) torsion balance. A detailed discussion of the weighing procedure can be found in the fourth paper of this series (Halevy 1956).

Stomatal aperture was determined by the infiltration method, with kerosene, using the numerical scale established by Oppenheimer and Elze (1941). This scale ranges from 0 to 80.

Evaporation was measured by means of a Piche evaporimeter hung up on the examined tree. Temperature and relative humidity were determined with ventilated psychrometer.

EXPERIMENTAL RESULTS

All the data relate to four experimental trees growing near each other. Every point of the transpiration curves represents the average of transpiration rates of 5—6 leaves of the same age group and similar exposure to light. Aaronsohn (1951) who also examined citrus leaf transpiration found that there are differences in the rate of transpiration on the different flanks of the trees. Our investigations were, therefore, restricted to the southern flank only.

The daily march of transpiration

On normal summer days we found two types of transpiration curves, The first (Halevy 1956, Figure 4) shows gradual increase of the losses to the main peak at about noon, coinciding with the peak in the meteorological conditions (heat and dryness of the air). In the morning and in the afternoon there are two minor peaks. Between these and the

noon peak occur depressions accompanied by reductions in the width of stomata. The second type is similar to that described by Oppenheimer and Mendel (1939, Figure 17). In sun leaves of the latter type, the first peak which occurred in the morning was followed by a drop in the rates of transpiration at noon; whereupon a second peak was reached in the afternoon, to be followed by the final evening drop. The rates of transpiration at the two peaks were similar, with one or the other attaining somewhat higher values. The transpiration curve of shade leaves shows only one peak which occurs at noon. As far as Sharav conditions are concerned, a distinction has to be made between a one-day Sharav and a Sharav lasting two or more days.

Figure 1 (May 5, 1953) shows the transpiration on the first of three successive Sharav days. The general march of the transpiration resembles that of a normal summer day. The rates of transpiration rise from the morning to a first peak coinciding more or less with the meteorological peak (about 10 a.m.). This peak usually occurs earlier and rises higher than on normal days. The highest transpiration rates recorded were those of the first peak on the first day of Sharav. The first peak is followed by a very sharp decrease in transpiration. Shortly afterwards, even though the unfavourable meteorological conditions continue unaltered, the transpiration curve rises to a second peak which is invariably lower than the first one. The daily curve terminates in the evening drop.

The behaviour of shade leaves is entirely different. The rate of transpiration rises gradually to a single noon-time peak. The water losses remain at the same level for several hours, and then — earlier than in sun leaves — comes a sudden fall towards the evening. The mid-day decrease in transpiration, which is typical of sun leaves, is absent in shade leaves. The transpiration rate of shade leaves is therefore higher at noon than that of sun leaves.

The Sharav which prevailed on the day of the investigation related above, continued throughout the night and the following day. May 6th, 1953, on which the transpiration values of Figure 2 were recorded, can thus be regarded as typical for a second successive day of Sharav. It can be seen that the transpiration of sun leaves is rather high from the early morning. It goes up rapidly to a first peak around 8 to 9 a.m. In the three instances of investigations carried out on successive Sharav days, the morning peak on the second day of Sharav was lower than that of the first day. Past the morning peak, the rate remained high for about two hours, and then followed a sharp drop. The rate of transpiration at noon often dropped to rates even lower than those of the very early morning hours. This drop was accompanied by an almost complete closure of the stomata. This situation persisted for two or three hours. Then the stomata reopened a little and the transpiration rose to a second peak, lower than the first one. The curve terminated in the usual fall before sun-set. The behaviour of shade leaves is essentially the same as on the preceding day, except around noon, when the discrepancy between the transpiration rate of sun leaves and shade leaves is even greater.

On two occasions (April 24, 1953; June 6, 1953) a somewhat different curve was obtained (Figure 3). It shows three peaks; one — the highest — in the morning, the second at noon and the third in the afternoon.

Transpiration and the stomata

The curves of stomatal opening showed, in most cases, daily trends parallel to those of transpiration. The decrease of transpiration at noon was always accompanied by stomatal closure. On Sharav days, the stomata were shut almost throughout the day, and this undoubtedly lessened the rate of transpiration. But in spite of the fact that the fall of transpiration was usually found in leaves whose stomata were almost shut, there were many cases in which we found low transpiration rates even though the stomata were rather wide open. On the other hand, leaves with halfclosed stomata often showed high water losses. It may be concluded that, under the conditions of our studies, stomatal closure constituted the main factor in the control of transpiration only when the stomata were almost entirely closed; while smaller reductions in the size of the stomata had frequently no effect on the transpiration rate.

Total daily losses

In Table I, the daily totals of transpiration per leaf surface are given for ten days of experimentation.

TABLE I

Daily transpiration losses per unit of leaf surface (mg/dm.d) of Shamouti orange leaves

Date (1953)	Mature leaves			Young leaves		
	Sun leaves	Shade leaves	Relative transpiration of shade leaves (sun leaves = 100%)	Sun leaves	Shade leaves	Relative transpiration of shade leaves (sun leaves=100%)
Summer days						
May 8	13.820	8.665	64	12.240	8.050	66
May 28	14.740	8.765	59	13.150	8.210	62
June 19	15.210	9.230	61	12.970	8.250	64
Sharav days						
April 23	13.750	9.840	72	13.960	8.620	62
April 24	11.245	8.230	73	10.050	7.900	78
May 4	12.170	8.140	66	10.430	6.625	63
May 5	11.310	7.830	69	9.950	7.240	73
May 6	9.105	—	—	8.880	—	—
June 13	12.780	9.485	74	10.190	7.645	75
June 16	14.050	11.220	80	12.170	10.440	86

It can be seen that the total water loss on Sharav days is not greater than on normal summer days, and usually even smaller by 10 to 20%. These findings agree with the results of Oppenheimer and Mendel (1939), whose examinations were confined to a single true Sharav day.

As already mentioned, measurements on two successive Sharav days showed, on three occasions, smaller losses on the second day as compared with the first. In one case, transpiration was examined on three successive Sharav days (May 4—6, 1953) and it was found that the highest transpiration loss occurred on the first day and the lowest on the third. It is noteworthy that these results were obtained in spite of the fact that the Sharav conditions on the 5th and 6th of May were more extreme than on the 4th.

Transpiration of sun leaves and shade leaves

The daily march of transpiration in sun leaves and shade leaves has been described above. It has been shown that the fluctuations in the transpiration of shade leaves were smaller than those of sun leaves. This applies not only to the general march of transpiration, but also to transpiration curves of individual leaves. While the points of the sun leaf curves were plotted from averages of transpiration rates which often varied widely (the biggest loss being sometimes twice as much as the smallest), the fluctuations of the transpiration rates of individual shade leaves were usually relatively small. It is suggested that the variations in sun irradiation were largely responsible for the differences in the transpiration rates, besides the individual variations in the water balance of individual leaves. This assumption seems to be borne out by the results obtained on a cloudy autumn day (Halevy 1956) when agreement between the single transpiration rates was of a high order even in sun leaves. The daily transpiration per surface unit of shade leaves was always lower than that of sun leaves. But while the differences on normal summer days were around 40% of the sun leaf values, they amounted only to some 20—30% on Sharav days. This was undoubtedly brought about by the intensive transpiration of shade leaves at mid-day (see Figures 2 and 3). In this respect, there is no difference between young and mature leaves.

Transpiration of young and mature leaves

The bulk of the investigation was carried out between April and June. At that period, a considerable proportion of the foliage consisted of full-size young leaves from the latest growth cycle.

TABLE II

The daily transpiration of young leaves in relative figures (mature leaves = 100%)

Date (1953)	Sun leaves	Shade leaves
May 8	89	93
May 28	89	94
June 18	85	89
April 23	102	88
April 24	89	96
May 4	86	81
May 5	87	92
May 6	97	—
June 13	80	81
June 16	86	93

Table II is based on the data of Table I. It can be seen that, both in the sun and in the shade, the total daily transpiration of the young leaves was smaller than that of the mature leaves. It is also noteworthy that the differences between young and mature leaves tend to be smaller in the shade than in the sun. A comparison of the daily transpiration curves indicates that the trends outlined above are largely due to the differences in transpiration during the late morning hours. As an example, the transpiration curves

of young and mature leaves are given for May 8, 1953, as recorded in the sun (Figure 4) and in the shade (Figure 5), together with the curves of stomatal opening.

It seems that the stomata of the young leaves are more sensitive to light as well as to inner water deficits. Although the opening of the stomata in the morning is faster in the young leaves, the resulting increase in evaporation is slight, since the meteorological conditions at the time of the day do not favour high evaporation rates. The total daily transpiration of young leaves is considerably more affected by their greater sensitivity to inner water deficits, on account of which the stomata close one or two hours before those of mature leaves. This tendency is more pronounced in shade leaves. It is also possible that the cuticular transpiration of young leaves is greater than that of mature leaves. Anyhow, under the conditions of our investigations, the early closure of the stomata at noon had a decisive influence on the total daily transpiration.

With a view to collecting more data on this subject, *ad hoc* measurements were made on two consecutive normal spring days (April 19—20, 1953) with sun leaves only, and the leaves were classified according to their age, in the following manner:

Type I: Small, immature leaves. This group includes leaves about 2 to 3 weeks old. Since at the end of April the leaves of the normal spring growth cycle are no longer of this type but larger and more mature, several branches of two trees were pruned about three weeks in advance of the weighing, to promote the production of immature leaves suitable in time for the investigation.

Type II: Young, full-grown leaves. These are the leaves of the spring growth cycle, 2—2½ months old. Although having mostly attained their final size, they have as yet retained a light-green colour. All the "young leaves" in Table I and in Figures 4 and 5 belong to this group.

Type III: Mature leaves. Dark-green leaves of the preceding growth cycles.

Type IV: Old leaves. Leaves approaching the stage of abscission. Their colour, at least in part, is yellowish-green, as a consequence of the decomposition of the chlorophyll.

The determination of transpiration of 128 leaves included in the experiment was carried out in a sequence of two leaves of types II and III followed by one leaf of types I and IV. The results are presented in Table III.

TABLE III

Transpiration of leaves of various age-groups

Leaf type	Average transpiration in mg/g.h (milligrams per gram fresh weight and hour)	Relative average transpiration (type III=100%) (%)
Type I: Small, immature leaves (24)*	262	122
Type II: Young, full-size leaves (43)	181	85
Type III: Mature leaves (43)	214	100
Type IV: Old leaves (18)	128	60

* The figures in brackets indicate the number of leaves examined.

The results demonstrate clearly that the very young leaves reached the highest transpiration figures. Although cuticular transpiration undoubtedly acts as an important factor, the high transpiration rate of the leaves is at least partly due to the slow reaction of the stomatal closure mechanism. It was frequently observed that during the hottest hours of the day, when the stomata of the leaves of other types were closed, the stomata of the immature leaves remained open. The reduced transpiration of the leaves of type II, as compared with those of type III, is in agreement with results previously discussed (Table II). The lowest rates of transpiration were found in the old leaves, due — at least in part — to the defective response of their stomata, many of which had altogether lost the ability to open.

DISCUSSION

Transpiration on Sharav days

Our investigation corroborates the conclusion of Oppenheimer and Mendel (1939) that transpiration losses on sirocco days were not found to be much higher than losses corresponding to the general conditions of the season, while evaporation during the day time was found on such days to be about 60% higher than normal. We wish to add that we were able to confirm these statements on Sharav days much hotter and drier than those under which the above authors operated. On two days (June 16 and April 23, 1953) the evaporation was about three times higher than on a normal day; yet in spite of that, the daily transpiration losses were not higher than on other days. A comparison of the curves of transpiration with those of stomatal aperture proves convincingly that the main regulating mechanism of transpiration is the closure of the stomata. It seems that the main reason for this closure is the water deficit of the air. A clear correlation between stomatal closure and the dryness of the air was found in apple trees by Magness and Furr (1930) and by Furr and Degmann (1932).

The efficiency of the stomatal mechanism in citrus leaves was proved by Haas and Halma (1932) and afterwards by Oppenheimer and Mendel (1939), and Mendel (1945, (1951).A close relation between transpiration and stomatal opening was found by Poljakoff (1946) and Oppenheimer (1947) in some Mediterranean trees, by the latter author in maize (1951), by Thomas (1949) in tropical plants and by van der Paauw (1954) in some field crops.

But in spite of the general agreement of the daily march of transpiration with that of the stomatal movements, we often found decreases in transpiration without a corresponding closure of the stomata. Similar phenomena have been described by Oppenheimer (1951) in maize and by Shmueli (1953) in banana and in maize. The curves of transpiration on Sharav days are similar to those described by Stocker (1935) and interpreted as typical for conditions of water shortage: a pronounced 'ultramaximum' in the morning, followed by a deep fall in transpiration shortly afterwards.

Before we discuss the influence of the sharav on citrus trees, and compare it to similar phenomena in other countries, we wish to stress that, although there are hot and dry winds in many parts of the world, almost each one of them has its own

specific character, and they should not be compared without certain qualifications. This is true with respect to the Foehn in Switzerland, the Sirocco in Italy and French North Africa (which is often hot and wet), the Sukhovey in southern Russia, the Santa Anna and Northers in California, and in our region — the Sharav (Khamsin) and the Sharqiyeh. Boeuf and Genet (1906) reported from Tunis and Reed and Bartholomew (1906) from California, extensive damage to citrus trees caused by such dry winds, which took the form of defoliation, death of twigs and loss of fruit. The death of the leaves and twigs becomes sometimes apparent before the end of the first day of the desiccating wind. We did not notice anything like it even on the worst Sharav days, with 5 to 7% R.H. and 39°C in the shade; whereas the observations of Reed and Bartholomew relate to R.H. of about 25% and temperature around 32°C. We assume that the differences in the reaction of the trees to these hot days must be attributed to the fact that, while in the Sharav irradiation is weak and there is almost no wind, the wind velocity associated with the Californian Northers is from 15 to 60 miles per hour. Reed and Bartholomew (1930) found transpiration rates during the dry windstorms much higher than under normal weather conditions. Results similar to ours, i.e. low transpiration rates under hard meteorological conditions, were found by various authors, working with trees in different countries. Oppenheimer (1932, 1947) found in some Mediterranean trees very low transpiration rates, approaching zero on summer and Sharav days, due to complete closure of the stomata. Examining various species of trees in Natal, Bruckner (1945) found lower transpiration rates than usual during dry weather. We may argue that citrus trees bear a xerophytic character. According to Maximov (1931), low transpiration rates under normal conditions are not characteristic of xerophytic plants, since, as a rule, their transpiration is then rather high. But a true criterion of xerophytic species is their ability to reduce their water loss to a minimum under conditions of strees. In spite of the general agreement of our results with those of Oppenheimer and Mendel (1939) and Mendel (1945), there are marked differences in the magnitude of the evaporation rates. Although the data of these authors are presented in mg/g.h and ours in mg/dm^2.h, we have found that the average area of 1 gram leaf is about 34 cm^2, so that conversion of figures calculated per gram of dry weight into values related to a square decimeter, and *vice versa*, is possible. While the above workers found — on summer days — daily transpiration totals of 15 to 28.8 gm per 100 cm^2, we found losses of 9 to 15 gm only. This holds true also for individual transpiration rates. While Oppenheimer and Mendel often found rates above 1000 mg/g.h, and Mendel recorded on one occasion as much as 1700 mg/g.h, our highest rate was 862 mg/g.h and even such rates were very rare. We assume that the differences are due to the different method of weighing. Before we observed the phenomenon of stomatal opening after plucking of the leaf (Halevy 1956) we, too, exposed the leaves for 2 to 3 minutes between the initial and the final weighing, and found, as a rule, higher rates than afterwards with our improved technique. Bartholomew (1931), who examined the transpiration of citrus leaves by a different method (attaching hermetically closed vials with $CaCl_2$ to both sides of the leaf), found transpiration rates lower

than those of Oppenheimer and Mendel, and rather similar to ours. The same is true of the rates recorded by Bialoglowski (1934) for lemon cuttings under controlled conditions. Oppenheimer (1952), basing his calculations upon his transpiration data for summer days, tried to obtain an approximate picture of the water consumption of one dunam of an orange grove, assuming a certain number of leaves per tree. Later on, thanks to an exact counting of the leaves of a Shamouti orange tree (Monselise and Heymann-Hershberg 1953), it became apparent that the real number of leaves is much greater, and this would imply losses per dunam which appear exaggerated. It thus appears that the transpiration rates of Oppenheimer and Mendel are too high, probably as a consequence of their manner of weighing.

Transpiration of sun and shade leaves

Higher transpiration rates of shade leaves as compared with sun leaves, around mid-day, curious as they may appear at first sight, were also found by other workers. Oppenheimer and Mendel (1939) met this situation on the single Sharav day they carried out examinations (p. 219, Figure 20). Pisek and Tranquillini (1951), working with *Picea excelsa*, found that on days with high saturation deficits of the air the transpiration on the shaded flank of the tree was higher than on that exposed to the sun. Nutmann, working with *Coffea arabica* (1941) and with *Eugenia aromàtica* (1953), discovered that, at noon, when the stomata of sun leaves close, the stomata of shade leaves remain open, and therefore shade leaves transpire during these hours much more than sun leaves. The same was found also by Lemée (1947) in the case of *Teucrium scorodonia.*

Transpiration of young and mature leaves

Many workers have studied the differences in the transpiration of young and mature leaves, but arrived at different conclusions. With regard to citrus, Oppenheimer and Mendel (1939) and Bialoglowski (1934) found that, in most cases, young leaves lose more water than mature ones. In contrast to their findings, Bartholomew (1931) and Reed and Bartholomew (1930) arrived at the conclusion that the young, light-coloured leaves of citrus transpire less than mature leaves. The latter authors and, earlier, Boeuf and Genet (1906) maintained that this was the reason why young leaves were less damaged by desiccating winds than older mature leaves. Our investigations are in line with the conclusion of the latter authors. However, the possibility should be taken into account that different investigators call the same leaf by different names. We therefore examined the transpiration of four well defined leaf types differing in their age and structure. Reed and Hirano (1931) found that the density of the stomata per unit of leaf surface is greatest in very young leaves at the end of the period of tissue differentiation in the epidermis. This may be one of the reasons why stomatal transpiration is greatest in this group of leaves, in addition to relatively large cuticular transpiration. We found that the stomata of young leaves are more sensitive than those of older stages and therefore their regulation is more effective. Boeuf and Genet

(1906) explain the superior regulation in young leaves by assuming that the stomata of young leaves are more plastic and this permitted them to close more perfectly when the leaves were exposed to drought. As to old leaves, Bartholomew (1931) found, like ourselves, that the stomata of such leaves are sometimes inactive and remain constantly closed.

ACKNOWLEDGMENT

The author is very obliged to Prof. H. R. Oppenheimer who suggested this work and helped in the editing of the present English summary. Thanks are also due to Dr. S. P. Monselise for his guidance and advice.

REFERENCES

1. Aaronsohn, A., 1951, The influence of light intensity on the structure of citrus leaves, Unpublished M.Sc. thesis, Faculty of Agriculture of the Hebrew University.
2. Ashbel, D., 1934—5, Der Scirocco Palaestinas, *Folio Internae orientalia*, Jerusalem.
3. Ashbel, D., 1935, The characteristics of the Sharav (Khamsin) in Palestine (in Hebrew), *Hatteva' ve-Haaretz*, **3**, 293—299.
4. Ashbel, D., 1937, Evaporation in Palestine, *Palest. J. Bot. hort. Sci.*, **1**(3), 8—26.
5. Bartholomew, E. T., 1931, Certain phases in citrus leaf transpiration, *Amer. J. Bot.*, **18**, 765—783.
6. Bialoglowski, J., 1934, The saturation deficit as a factor in the rate of transpiration, Ph.D. thesis, University of California.
7. Boeuf, F. and Genet, P., 1906, Le dessèchement des orangers à la suite de scirocco d'automne, *Rev. Hort. Tunis*, **5**, 20—27, 50—58 (cited from Reed and Bartholomew 1930).
8. Brueckner, A. E., 1945, Transpiration studies of some Natal midlands Thornveld trees, *S. Afric. J. Sci.*, **41**, 186—193.
9. Furr, J. R. and Degman, E. S., 1932, Relation of moisture supply to stomatal behaviour of apple, *Proc. Amer. Soc. hort. Sci.*, **28**, 547—551.
10. Haas, A. R. C. and Halma, F. F., 1932, Relative transpiration rates in citrus leaves, *Bot. Gaz.*, **93**, 466—473.
11. Halevy, A., 1956, Orange leaf transpiration under orchard conditions. IV. A contribution to the methodology of transpiration measurements in citrus trees, *Bull. Res. Counc. of Israel*, **5D**, 155—164.
12. Huber, B., 1927, Zur Methodik der Transpirationbestimmung am Standort, *Ber. dtsch. bot. Ges.*, **45**, 611—618.
13. Lemee, G., 1947, Recherches sur l'économie de l'eau des formes d'ombre et de lumière du *Teucrium scorodonia*, *Rev. gén. Bot.*, **54** (639), 105—137.
14. Magness, J. R. and Furr, J. R., 1931, Stomatal activity in apple leaves, *Proc. Amer. Soc. hort. Sci.*, **27**, 207—211.
15. Maximov, N. A., 1931, The physiological significance of the xerophytic structure of plants, *J. Ecol.*, **19**, 273—282.
16. Mendel, K., 1945, Orange leaf transpiration under orchard conditions. II. Soil moisture content decreasing, *Palest. J. Bot. Rehovot*, **5** (1), 59—87.
17. Mendel, K., 1951, Orange leaf transpiration under orchard conditions. II. Prolonged soil drought and the influence of stocks, *Palest. J. Bot. Rehovot*, **8** (1), 45—54.
18. Monselise, S. P. and Haymann-Hershberg, L., 1953, Influence of exposure and age on dry matter content, area and mineral composition of Shamouti orange leaves, *Proc. Amer. Soc. hort. Sci.*, **62**, 67—74.
19. Nutmann, F. J., 1941, Studies in the physiology of *Coffea arabica*. III. Transpiration rates of whole trees in relation to natural environmental conditions, *Ann. Bot.*, **59**, 82.

20. Nutmann, F. J., 1953, Note on the relationship between climatic factors and transpiration and assimilation of *Eugenia aromatica*, *Ann. Bot.*, **17** (68).

21. Oppenheimer, H. R., 1932, Zur Kenntnis der hochsommerlichen Wasserbilanz mediterraner Gehoelze, *Ber. dtsch. bot. Ges.*, **50a**, 185—245.

22. Oppenheimer, H. R., 1947, Studies on the water balance of unirrigated woody plants, *Palest. J. Bot. Rehovot*, **6** (1), 63—77.

23. Oppenheimer, H. R., 1951, Physiological behaviour of maize under irrigation, *Palest. J. Bot. Rehovot*, **8**, 32—44.

24. Oppenheimer, H. R., 1952, The irrigation of citrus trees (in Hebrew), in *The Theory of Irrigation and Irrigated Plants*, Ziv, D., *Hassadeh Library*, Tel-Aviv, 331—349.

25. Oppenheimer, H. R. and Elze, D. L., 1941, Irrigation of citrus trees according to physiological indicators, *Palest. J. Bot. Rehovot*, **4**, 20—46.

26. Oppenheimer, H. R. and Mendel, K., 1939, Orange leaf transpiration under orchard conditions. I., *Palest. J. Bot. Rehovot*, **2**, 171—250.

27. van der Paauw, F., 1954, Limiting factors of transpiration, *VIIIeme Cong. Intern. Bot., Paris*, Sec. 2 et 12, 252—255.

28. Pisek, A. and Tranquillini, W., 1951, Transpiration und Wasserhaushalt der Fichte bei zunehmender Luft- und Bodentrockenheit, *Physiol. Plant.*, **4**, 1—27.

29. Poljakoff, A., 1946, Ecological investigations in Palestine. I. The water balance of some Mediterranean trees, *Palest. J. Bot. Jerusalem*, **3** (3), 138—150.

30. Reed, H. S. and Bartholomew, B. T., 1930, The effects of desiccating winds on Citrus trees, *Bull. Univ. Calif. agric. Exp. Sta.*, **484**, 59 pp.

31. Reed, H. S., and Hirano, E., 1931, The density of stomata in citrus leaves, *J. agric. Res.*, **43**, 209—222.

32. Shmueli, E., 1951, Irrigation studies in the Jordan Valley. I. Physiological activity of the banana in relation to soil moisture, *Bull. Res. Counc. of Israel*, **3**, 228—247.

33. Stocker, O., 1935, Transpiration und Wasserhaushalt in verschiedenen Klimazonen. III., *Jb. wiss. Bot.*, **81**, 464—486.

MAL SECCO OF CITRUS IN ISRAEL AND NEIGHBOURING COUNTRIES

I. Reichert and M. Chorin
Agricultural Research Station, Rehovot

ABSTRACT

The characters distinguishing Mal Secco — which has been present in Palestine since before 1929 — from anthracnose and blast are discussed. Isolations from freshly infected, red-tinted wood invariably delivered *Deuterophoma tracheiphila*, while from older branches *Colletotrichum gloeosporioides* was also isolated. Such mixed infection probably explains previous authors' erroneous belief that the latter fungus produces Mal Secco.

The disease spreads at temperatures below 30°C; humid conditions favour infection, while windbreaks and grafting on orange stock reduce the danger. Summer pruning and the use of resistant lemon varieties, like Monachello, are recommended.

The Mal Secco in lemons and citron was discovered in Palestine and neighbouring countries in 1929 (Reichert and Fawcett 1930), but it is quite certain that it had been present in the area long before that year, since the typical failure in lemons and citrons had been noted by veteran growers much earlier. After 1930, more attention was given to the disease as its effects were becoming increasingly serious. Since then no noticeable recovery has taken place. The neglect during the war years contributed its share to a further aggravation of this disease. As we are now witnessing a considerable interest in new lemon planting in this region, it might be worthwhile to review the condition of this disease as we have observed it in Israel, Syria (1934), Southern Turkey (1934), and Cyprus (1935, 1956). It should be noted that it has not been found in Egypt. The results of our investigation might assist in clarifying the controversy raised by Pasinetti (1952) regarding the causative agent of Mal Secco.

Symptoms

The most frequent manifestation of the disease in this region consists in a sudden wilting, and then a yellowing and subsequent shedding of the upper leaves; this leading finally to a dieback of the supporting branches, of major limbs, and ultimately of the whole crown (Figure 1). The malady shows a tendency to attack only half the branches, descending from the top as a more or less broad brownish strip. A sure sign that the dieback is due to Mal Secco and not to some other disease, is the reddish discoloration of the wood of the younger and still green branches (Figure 2). At a later stage, this reddish colour changes to a darker brown. Though the downward trend in the morbidity pattern, as described above, is most common, it appears that the disease may occasionally begin at the roots.

The Mal Secco has often been confounded with two other diseases occurring in the Eastern Mediterranean region, Anthracnose caused by the fungus *Colletotrichum*

Received April 25, 1956.

gloeosporioides and the Blast caused by a bacterium, *Phytomonas syringae*. These two diseases cause a dieback of the upper and of the side branches. Originally all these diseases were confused and called by Savastano (1921) 'Mal Secco'. It was Petri (1929) who differentiated the three diseases by discovering their separate pathogens and by describing their individual symptoms. Externally these diseases may, in fact, be distinguished quite easily. The branches affected by Blast never give rise to any fruiting bodies on infected portions such as are associated with Anthracnose and Mal Secco. The Blast infection is usually accompanied by exudation of gum droplets from the affected portions. Anthracnose and Mal Secco fruiting bodies often appear simultaneously on the same morbid portions. The fungus fruiting bodies found with the Mal Secco disease are covered with a greyish thin epidermis, and, where they break off from the epidermic covering, they spread irregularly without an indication of any regular pattern. The fruiting bodies of Anthracnose are usually found free of the epidermic covering, and tend to form into concentric arrangements. The most reliable method for distinguishing the fruiting bodies consists in microscopic observations. The fruiting bodies of the *Deuterophoma tracheiphila* are pycnidia with a roundish collar and with minute spores without conidiophores (Figure 3), whereas the fruiting bodies of *Colletotrichum gloeosporioides* are open and concave, and are furnished with a stromatic base supporting spore-bearing conidiophores.

Causal agent

The causal agent of Mal Secco may be easily isolated from green branches which show the initial stage of the disease in the form of a red-tinted wood. Isolation from older branches is considerably more difficult, since it tends to be confounded by the presence of *Colletotrichum gloeosporioides* within the same infected area. It is very likely that this contamination may have led investigators like Pasinetti (1952) to consider *Colletotrichum gloeosporioides* as the primary pathogen. It is noteworthy that all our isolations from reddish tinted tissues of Mal Secco affected branches gave pure cultures of *Deuterophoma tracheiphila*. Furthermore, positive infections were successfully achieved with these isolations. Pure cultures produced mostly pycnidia, but at times we also observed hyphae with lateral branches bearing at their tips *Acrominium*-like spores. Most of our isolates were chromogenic, though a whitish culture would occasionally develop.

Similar isolations were also made by us from material collected in Cyprus and South Turkey (Alexandretta, 1935). We received specimens for examination from Dr. Jen, the Turkish plant pathologist, and we were able to isolate the same fungus. (The fungus resides in the wood vessels of the tree and may be easily detected on microscopic sections stained with cotton blue.) The pycnidia in our examinations measured 84—105 μ in diameter, with the majority around 90 μ. The length of the collar which fluctuated between 42 and 50 μ, averaged 45 μ. The spores measured 1—3.5 μ in length and 1—1.5 μ in width, the mode being 2.5 μ.

Contributing conditions

It has been established by Sereni (1935) that 18°C is the optimum temperature for the growth of *Deuterophoma tracheiphila*, while 3° and 30°C represent the minimum and maximum temperature respectively. Although at 32°C there was no growth, the spores were not destroyed. At 35°C the spores lost their germination power after 5 days; at 40°C — after 3 days. Sereni (1931, 1935) also found that above 24°C the chromogenic isolates lost the ability to form pigments. The pigment was more pronounced in liquid than in solid media. Similar results were obtained in Italy by other workers (Petri 1930, Goidanich and Ruggieri 1953).

The fact that spores do not germinate at temperatures higher than 30°C may explain the fact that no new infections of branches are to be found in the summer. As the disease develops during spring and autumn, i.e. during the wet season, it may be assumed that humid conditions are also favourable for its development. Though accurate experiments regarding the effect of humidity have not as yet been carried out, it seems that humidity represents a factor of secondary importance.

Wind is of major importance in the spreading of the disease, since (a) the pycnidia are windborne, and (b) the rubbing of branches against each other by wind movement causes abrasions which facilitate the entry of the fungus. The importance of winds is clearly borne out by the fact that groves with effective windbreaks suffer considerably less damage from Mal Secco than those which are more open to wind incursions. It has also been observed in Cyprus lemon groves that individual trees exposed to wind action are conspicuously more affected by the disease (Figure 4).

Observations in Israel and Cyprus suggest that the rootstock may play a decisive role in determining the rate of decline of lemons due to Mal Secco. Lemons grafted on sweet lime and on rough lemon show greater susceptibility and are subject to a more rapid decline than those grafted on bitter orange. A survey of a lemon grove carried out in Palestine in 1929 (Reichert 1933) revealed that 68% of infected trees were grafted on sweet lime and only 19% on bitter orange. Similar results were obtained in three other groves.

Control

(1) The best control of Mal Secco consists in the pruning of infected parts and thereby eliminating the immediate sources of infection. In planning the pruning operations, it should be borne in mind that the infective activity of the pathogen ceases temporarily during the summer months. The best time for pruning is therefore before the wet autumn season sets in, i.e. in September and October.

(2) Next in importance is the use of resistant varieties of lemon. Of the three lemon varieties grown in Israel, the local variety, Eureka and Lisbon, the first two have been found to be more susceptible. True resistant varieties of lemon have been found in Italy: Monachello and Interdonato (Petri 1932, Ruggieri 1948). Buds of these varieties which were kindly sent to us in 1938 by the late Professor Petri (Reichert 1953) were planted in the same year at two places, Na'an and Sarafand. Up to now the trees appear

to thrive in spite of the fact that they show slight infection. Interdonato cannot be considered as a commercial variety, since the tree bears poorly and the fruits are much too large for the market. The Monachello, however, bears well and produces good sized fruits.

(3) In view of the effectiveness of winds in spreading Mal Secco, strong emphasis should be placed upon the construction of adequate windbreaks. This applies especially to orchards situated near the sea and on hill sides. Each 100—200 metres, in accordance with the frequency and velocity of winds, should be provided with a wind screen.

(4) The question of rootstock selection should also be considered. Sweet lime and rough lemon are both very susceptible to Mal Secco. This vulnerability may well be related to the susceptibility of these stocks to xyloporosis. It is a fact that the stem-pitting due to xyloporosis is always found in trees declining with Mal Secco. As long as no better commercial stocks resistant to Mal Secco are available, bitter orange is the only stock we can recommend. One might suppose that the sweet orange stock, which in itself is quite immune to both Mal Secco and xyloporosis, would make a better stock for lemons, but according to Dr. Petri's experience (written communication, 1938), no better results have in fact been obtained with sweet than with bitter orange. Additional experiments are necessary to clear up this problem.

An interesting approach is made by some Italian investigators, notably Ciferri (1941), who recommend sandwich grafting which would employ, for instance, bitter orange as rootstock, a resistant variety (sweet orange or mandarin) as the middle portion, and lemon as the final graft. However, in Israel, where grapefruit (also resistant to Mal Secco) was used as the middle graft on bitter orange, the results have not been particularly promising, as 21% infection was found after two years. Further investigations on the effectiveness of sandwich grafting are undoubtedly required.

REFERENCES

1. Ciferri, R., 1941, *Manuale di Patologia Vegetale*, p. 616.
2. Goidanich, G. and Ruggieri, G., 1953, Il Mal Secco degli agrumi, *G. Agric., Jesi*, **63**, p. 21—22.
3. Pasinetti, L., 1952, Sulle vere cause determinanti il 'Mal Secco' degli agrumi e su nuovi orientementi terapeutici, *Ann. Fitopatol.*, **1**, 2—67.
4. Petri, L., 1929, Batteriosi dei rametti e mal del secco dei limoni in Sicilia; *Boll. Staz. Pat. veg. Roma*, **9**, 1—11.
5. *Idem*, 1930, Ulteriori richerche sulla morfologia, biologia e parasitismo della *Deuterophoma tracheiphila*, *ibid.*, **10**, 192—221.
6. *Idem*, 1932, Lotta contro il Mal Secco dei limoni, *G. Agric. merid.*
7. Reichert, I., 1933, Death of lemons and citrons, *Bustenai*, **9**, 84—89 (Hebrew).
8. *Idem*, 1953, Lionello Petri; *Palest. J. Bot. Rehovot*, **8**, 224—227.
9. Reichert, I. and Fawcett, H. S., 1930, Citrus diseases new to Palestine, *Phytopathology*, **20**, 1003.
10. Ruggieri, G., 1948, Fattori chei condizionano o contribuiscono allo sviluppo del 'Mal Secco' dei agrumi e metodi di lotta contro il medesimo, *Ann. Sper. agr.*, n.s., **2**, 255—305.
11. Sereni, D., 1931, Perdita della facoltà germinativa delle spore di *Deuterophoma tracheiphila*, *Boll. Staz. Pat. veg. Roma*, No. 11, 6 pp.

12. *Idem*, 1935, unpublished notes.

13. SAVASTANO, L., 1921, Sulla gommosi secca o Mal Secco degli agrumi, *Boll. Staz. Agrum. Frutt. Acireale*, No. 42.

Figure 1. Eureka lemon on sour orange affected with Mal Secco. Upper branches drying up.

Figure 2. Cross section of sour orange trunk showing the peripheric discoloration of the wood.

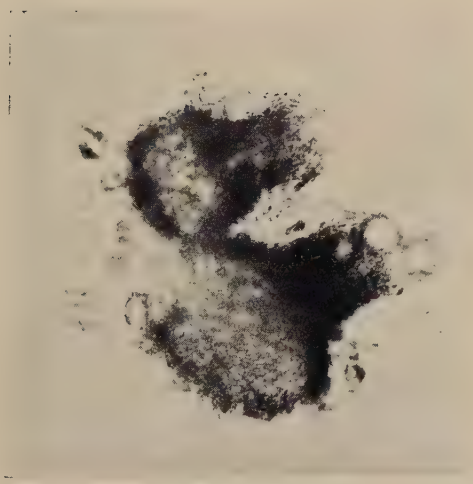


Figure 3. Pycnidia of *Deuterophoma tracheiphila*, longitudinal section. (×500).

Figure 4. Monachello lemon on sour orange resistant to Mal Secco.

THE EFFECT OF DIFFERENT FACTORS ON THE ASCORBIC ACID CONTENT IN CITRUS FRUITS.

II. THE RELATIONSHIP BETWEEN SPECIES AND VARIETY AND THE ASCORBIC ACID CONTENT OF THE JUICE*

ARIEH COHEN

Ramat Gan

ABSTRACT

(1) The vitamin C content of the juice of 62 varieties of 4 citrus species was determined. Oranges were generally found to be richer in ascorbic acid than grapefruit or lemon. The smallest amount was found in mandarins.

(2) It was noted in many cases that taxonomically related varieties have a similar ascorbic acid content. Crosses between two varieties of mandarins with a low ascorbic acid content inherit this character, while hybrids between mandarins and oranges have a distinctly higher vitamin C content. This might be regarded as evidence to support the assumption that Clementine is a hybrid between a mandarin and a sweet or a sour orange.

(3) Tests of lemons have indicated that proper bud selection might improve the vitamin C content of the juice.

(4) The Baladi variety, appearing as a bud-mutation on Shamouti trees, is richer in ascorbic acid than the Shamouti. The determination of this constituent may help to locate the place of mutation on the Shamouti tree.

INTRODUCTION

The purpose of this study was to compare the vitamin C content in different varieties of citrus fruits growing in Israel, and to ascertain whether there exists a relationship between the vitamin C content and the taxonomical position. The classification of the genus *Citrus* of Swingle, as adapted in the horticultural subdivisions by Webber (1943), has been followed throughout.

METHODS AND MATERIALS

Thanks to the kind co-operation of Dr. K. Mendel of the Division of Citriculture, we had the opportunity of examining during a period of three years the fruit from the collection of the Agricultural Research Station at Rehovot. This collection includes about 60 species and varieties, mostly grafted on sour orange rootstock. There are 1 to 5 trees to each variety. Composite samples of 25 fruits from each tree were examined. The grove is on light soil with a slope to the east, and was about 16 years old at the time of the examination.

* From a thesis submitted to the Hebrew University of Jerusalem in partial fulfilment of the requirements for the Ph.D. degree.

Received April 3, 1956.

Bull. Res. Counc. of Israel, Vol. 5D, 1956.

TABLE I

Average vitamin C content of citrus varieties grafted on sour orange rootstock, examined during the years 1947/49

(mg ascorbic acid in 100 ml juice)

Group	Variety	Ascorbic acid content*	
Orange			
Early varieties	Kinarti	58.7	(10)
	"Bizzaria"	59.5	(15)
	Norris Early	61.3	(3)
	Trovita	63.9	(10)
	De Nice	67.1	(14)
	Hamlin	68.2	(11)
	Viciedo	68.8	(13)
Midseason varieties	Shamouti	51.0	(19)
	Cadenas	55.2	(6)
	Baladi	55.8	(5)
	Parson Brown	63.3	(7)
	Magnum Bonum	64.0	(2)
	Hitmali	66.8	(10)
	Pineapple	78.4	(6)
Late varieties	Verna	58.5	(8)
	Lue Gim Gong	64.7	(8)
	Valencia	65.2	(5)
Blood oranges	Maltese Blood	56.7	(9)
	Ruby Blood	56.7	(12)
	Paterno	60.3	(6)
	Doppio Sanguigno	60.3	(5)
	Doppio Signorelli	63.7	(12)
	Sanguigno Semplice	64.3	(7)
Navel oranges	Washington Navel	61.1	(10)
	Sigillata	61.5	(9)
	Carter Navel	61.7	(8)
	Australian Navel	61.7	(5)
	Thompson	64.7	(5)
	Robertson	68.8	(7)
Grapefruit	Imperial	47.0	(4)
	Natsu-Mikan	48.5	(6)
	Thompson	49.2	(13)
	Marsh Seedless	49.6	(18)
	McCarty	50.3	(13)
	Triumph	51.0	(10)
	Foster	52.2	(12)
	Pernambuco	53.5	(2)
	Duncan	55.5	(12)
Lemon	Meyer	32.3	(9)
	Eureka	45.1	(9)
	Villafranca	51.1	(9)
Mandarin King	King	40.0	(5)
Satsuma	Wase	36.0	(4)
	Owari	33.0	(4)
Willow leaf	Oneco	48.5	(2)
	Fewtrell's**	40.0	(4)
	Avana	38.8	(15)
	Sicilian Giant	40.2	(16)
	Abu Sura	39.0	
	Zib	39.0	
Tangerines	Ellendale	39.0	
	Local Large Tangerine	37.0	
	Sylhat	41.0	
	Nagpur	34.5	
	Srinagar	44.0	
	Dancy	50.7	(26)
	Cleopatra	54.0	
	Clementine	64.7	(21)
Hybrids	Kara	34.0	
	Wilking	38.0	
	Kinnow	44.0	
	Temple	60.0	(22)

* The figure in parenthesis designates the number of examinations upon which the average is based.

** Grafted on rough lemon.

In addition we determined the vitamin C content in variety collections of the Government horticultural stations at Acre and Sarafand and of Deganya (Jordan Valley). The 8 year old grove at Deganya is on calcareous soil with a calcium carbonate content of approximately 35 percent. In this grove, every variety was grafted in one to three replicates on four different rootstocks, in plots consisting of three trees each. The results summarized in this paper were obtained from trees grafted on sour orange rootstocks, unless otherwise stated.

The vitamin C content of the juice was determined by titration with iodine, as we were primarily interested in comparative figures, and because, at the time of this study, indophenol was scarce in Israel. According to repeated tests, the results are approximately by 10 percent higher than the true ascorbic acid content, as determined by titration with 2,6-dichlorophenol-indophenol.

RESULTS OF THE EXPERIMENTS AND DISCUSSION

The numerous examinations of the vitamin C content of the juice of various citrus varieties have been summarized in Table I. Considerable differences in vitamin C content were found, values ranging from 20 mg% in Meyer lemon (determined 18.III.48) to 85 mg% in Pineapple orange (15.I.48).

Comparing the four citrus species examined by us, we see that *Citrus sinensis* has the highest vitamin C content, next come *C. limon* and *C. paradisi*, excepting the anomalous "Meyer" lemon. In the species *C. reticulata*, the vitamin C content fluctuates very considerably, but it should be borne in mind that this species is markedly heterogeneous also in other respects. The true mandarins have a low vitamin C content, which has also been found in Central America (Munsell 1949, 1950) and in Florida (Miller et al. 1941, Beacham and Bonney 1937, Harding and Sunday 1949).

No relationship was found between the vitamin C content of orange varieties and the five horticultural groups distinguished by Webber in this species, but significant differences were found between different varieties irrespective of the somewhat artificial horticultural classification. A survey and statistical evaluation of the analysis of the fruits from the Deganya collection are given in Table II. The vitamin C content of the Shamouti variety is significantly lower than that of the other orange varieties, and the juice of the orange varieties is poorer in ascorbic acid than that of the Clementine types. The same relationship between those varieties was also established at Rehovot under wholly different ecological conditions. The figures in Table II also indicate very clearly the higher vitamin C content in fruit from trees grafted on sour orange rootstock.

Although the fluctuation in vitamin C content of grapefruit varieties is far smaller than that of orange varieties, significant differences were nevertheless found when five grapefruit varieties in the collection at Rehovot were examined at two dates during the season 1949/1950. The figures in Table III show that, among the five grapefruit varieties examined, the seedy Duncan has the highest, and Marsh Seedless the lowest, ascorbic acid content. In both instances, the differences were shown to be highly

TABLE II

Ascorbic acid content of the juice of citrus varieties, budded on four rootstocks at Deganya B (mg ascorbic acid in 100 ml juice)

Variety	Rootstock				Average of variety
	Sour orange	Sweet lime	Rough lemon	Egyptian lime	
Shamouti	55.6	52.5	50.3	45.5	51.0
Bizzaria*	67.7	60.0	57.5	57.1	60.6
Kinarti	65.8	62.2	56.3	61.4	61.4
Washington Navel	65.1	58.4	58.1	56.3	59.5
Carter Navel	65.6	61.3	58.4	58.1	60.8
Sigillata**	63.1	67.5	62.4	56.5	62.4
Clementine	72.6	64.3	65.8	64.0	66.7
Clementine b***	69.9	68.7	65.1	65.0	67.2
Average of rootstock	65.5	61.3	59.1	57.9	

* A local name for a so far unidentified sweet orange variety. ** One missing plot estimated.
*** Clementine of different origin.

Analysis of variance

Factor	Sum of squares	Degrees of freedom*	Variance	*F*	Difference	
					significant	highly significant
Total	4461.60	77				
Varieties	1984.68	7	283.53	77.679	1.68	2.20
Rootstocks	675.50	3	225.17	61.690	1.19	1.56
Interaction:						
Rootstock × variety	1633.79	21	77.80	21.315		
Error	167.83	46	3.65			

* Two degrees of freedom lost due to missing plots.

significant in relation to three other varieties. A small but significant decrease in vitamin C content between December and February has also been established.

In every species there exists a considerable fluctuation in vitamin C content between the different varieties, but very similar contents are found in varieties closely related. Thus, for instance, in the group of navel orange varieties (these are mostly bud mutations of Washington Navel according to Webber 1943), the fluctuations are very small. Other examples of closely allied varieties, with a very similar vitamin C content, are: Sanguigno Semplice, Sanguigno Doppio, Signorelli; Valencia, Lue Gim Gong; Trovita, Washington Navel; Marsh Seedless, Thompson; Satsuma Owari, Satsuma Wase (Tanaka 1922); Avana, Sicilian Giant.

TABLE III

Ascorbic acid content of the juice of grapefruit varieties at Rehovot during the season 1949/1950 (*mg ascorbic acid in* 100 *ml juice*)

Variety	Date of examination		Average of variety
	22.XII.1949	3.II.1950	
Duncan	48.2	48.0	48.1
Foster	48.0	47.0	47.5
Thompson	46.8	45.5	46.1
McCarty	44.3	43.8	44.0
Marsh Seedless	44.0	43.0	43.5
Average of season	46.2	45.4	

Analysis of variance

Factor	Sum of squares	Degrees of freedom	Variance	*F*	Difference significant	Difference highly significant
Total	461.1	38				
Blocks	213.3	3	71.10	65.23		
Varieties	135.4	4	33.85	31.06	1.12	1.55
Season	5.4	1	5.40	4.95	0.71	0.96
Interaction: Block × variety	89.4	12	7.45	6.83		
Interaction: Season × variety	2.3	4	0.58	0.53		
Error	15.3	14	1.09			

From a systematical point of view, the mandarin group is a very heterogeneous one. It also shows very pronounced fluctuations in vitamin C content, as shown in Table I. Here the varieties have been arranged according to Webber's classification, which is based on morphological characters. The ascorbic acid content in the mandarin group is generally low and fluctuates between 33 and 51 mg in 100 ml juice. Also the hybrids which are crosses between two mandarins like Kara (King ♂ × Satsuma ♀), Kinnow (Willow Leaf ♂ × King ♀) and Wilking (Willow Leaf ♂ × King ♀) (Frost 1935) have a vitamin C content fluctuating between 34 and 44 mg%. A tangor, i.e. a cross between a tangerine and an orange, such as the hybrid Temple, stands out with its high ascorbic acid content. This is apparently due to the fact that one of the parents, the orange, has a considerably higher vitamin C content than the mandarin. The exceptionally high vitamin C content of the Temple variety in comparison with others of the mandarin group has also been stressed by Miller et al. (1941). It may be concluded that most true mandarins have a vitamin C content below 50 mg% and generally near to 40 mg%, and only due to hybridization with oranges does the ascorbic acid content rise above this level.

Trabut (Webber 1943) claims that the Clementine is an accidental cross between mandarin and sour orange. Webber, however, classifies this variety with mandarins and, according to his opinion, if it is a hybrid at all, then only a slight genetic influence of *sweet* orange may be assumed. The relatively high vitamin C content of the Clementine would seem to support the contention that it is not a true mandarin, but a hybrid, although we cannot draw any conclusions as to whether it is a cross with the sweet or the sour orange. Also in the case of the "Meyer" lemon, hybridization is responsible for a deviation from the level of vitamin C content prevailing in the lemon group. "Meyer" seems to be a cross between lemon and mandarin.

We also had the opportunity to examine the vitamin C content of Eureka lemons of an experimental plot at the Agricultural Research Station in Rehovot. Here the buds, taken from three different groves, had been grafted on both rough lemon and sweet lime, with every combination of bud and rootstock replicated in three plots. The results of vitamin C determinations, which were repeated seven times, are summarized in Table IV.

TABLE IV

Influence of bud origin and rootstock on vitamin C content of Eureka lemon juice (mg ascorbic acid in 100 ml juice)

Date of examination	Origin of bud	Magdiel		Tel Alon		Ramatayim	
	Root-stock	Sweet lime	Rough lemon	Sweet lime	Rough lemon	Sweet lime	Rough lemon
11.XII.1947		48	44	50	43	55	49
25.IV.1948*		59	56	57	56	59	58
20.IV.1948*		47	42	47	43	49	47
26.VII.1948*		50	47	50	44	59	49
21.IV.1949		47	41	46	42	50	46
19.V.1949*		53	54	55	53	53	57
1.III.1950		42	42	41	43	36	45
Average of rootstock		49.7	46.6	49.3	46.3	51.7	50.6
Average of bud		48.1		47.8		51.1	

* Summer lemons.

Analysis of variance

Factor	Sum of squares	Degrees of freedom	Variance	*F*	Difference significant	Difference highly significant
Total	5461.97	121				
Blocks	11.64	2	5.82	0.40		
Origin of bud	287.35	2	143.67	9.87	1.67	2.14
Rootstock	183.37	1	183.37	12.60	1.33	1.75
Season	3045.19	6	507.35	34.87		
Interaction:						
bud × rootstock	25.44	2	12.72	0.87		
bud × season	204.10	12	17.01	1.17		
rootstock × season	395.63	6	65.94	4.53		
Error	1309.25	90	14.55			

The differences between the strains appear to be small but consistent. The fruit originating from bud-wood taken in Ramatayim is richer in vitamin C than the fruit of Magdiel and Tel Alon provenance. In this connection it is interesting to note that the Magdiel trees are derived from Tel Alon bud-wood*. The results indicate that, under identical environmental conditions, differences in the vitamin C content may exist which may only be ascribed to the character of the bud-wood. It follows that the ascorbic acid content might conceivably be improved by suitable bud selection.

It is assumed that the Shamouti orange originated by bud mutation from the Baladi variety and it is also known that reverse mutations may appear on Shamouti trees (Oppenheim 1927). The Baladi fruit differs from the Shamouti in its roundish instead of oblong shape, rather numerous seeds and a higher vitamin C content (see Table I). Examining individual fruits on one and the same Shamouti tree, we found occasionally a sudden change in the vitamin C content of the juice about the middle of a branch, frequently accompanied by an increase in the number of seeds and a change of shape in some of the fruits. As an example, we bring in Table V the analysis of individual

* Personally communicated by Dr. K. Mendel.

TABLE V

Characters of individual fruits on the same limb of a Shamouti tree budded on sweet lime (Rehovot, 3.IX.1947)

Branch	Fruit No.	Weight (g)	Juice (%)	Composition of juice: Ascorbic acid (mg%)	Composition of juice: Citric acid (%)	Composition of juice: Total sol. solids (%)	Seeds*	Shape of fruit**
A	1	100	29.0	—	2.71	10.4	—	—
	2	121	24.0	51.4	2.65	10.2	—	—
B	3	124	28.6	56.0	2.67	10.2	—	—
	4	126	32.9	58.4	2.52	10.5	—	—
C	5	101	34.7	59.6	2.88	10.2	—	—
	6	108	31.9	56.3	2.40	10.5	—	—
	7	134	33.9	57.3	2.59	10.3	—	—
	8	70	30.0	55.2	2.85	11.3	—	—
D	9	126	31.4	55.2	2.53	10.3	—	—
	10	105	28.1	53.2	2.64	10.4	—	—
E	11	124	36.3	57.3	2.61	10.6	—	—
	12	132	34.8	56.5	2.45	10.3	—	—
	13	90	32.8	52.1	2.70	10.5	—	—
	14	95	34.2	65.3	2.85	10.3	+	+
	15	109	37.2	62.0	2.79	10.1	+	+
	16	117	34.6	63.5	2.82	10.2	—	—
	17	131	32.4	69.4	3.18	10.1	—	—
	18	105	31.9	71.9	3.00	10.4	+	—
	19	104	36.1	63.0	3.09	10.1	—	—
	20	108	35.2	62.2	3.00	10.2	+	+
	21	125	33.6	66.5	2.76	10.2	+	—
	22	131	38.9	62.3	3.04	10.2	—	—
	23	85	35.3	63.6	2.55	10.4	+	—
	24	91	36.3	63.5	2.67	10.4	+	—

* — = normal number of seeds
\+ = increased number of seeds

** — = normal oblong shape
\+ = roundish shape

fruits on one limb with 5 ramifications of a Shamouti tree grafted on sweet lime root-stock. The numbers of the fruits in the table indicate their position on the branch, starting at the base.

On branch E, an abrupt change in vitamin C content takes place between fruits 13 and 14. While fluctuations for the first thirteen fruits are between 51 and 59 mg%, for the fruits between 14 and 24 they range from 62 to 72 mg%. Beginning with fruit 14, many of the fruits contain an increased number of seeds. These phenomena may be explained by assuming that a reverse bud mutation to Baladi occurred on this branch between fruits 13 and 14. The possibility suggests itself that the analysis of vitamin C might be of assistance to the breeder in sorting out instances of Shamouti trees falling back to the original Baladi variety, without showing obvious changes in fruit shape.

ACKNOWLEDGMENT

The author's thanks are due to Prof. H. R. Oppenheimer for his guidance in these investigations, and to Dr. K. Mendel and Dr. L. Heymann-Herschberg for their kind co-operation in the execution of this work and for many valuable suggestions.

REFERENCES

1. Beacham, L. M. and Bonney, V. B., 1937, Ascorbic acid content of Florida citrus fruits, *J. Ass. Offic. agric. Chem.*, **20**, 517.
2. Frost, H. B., 1935, Four new citrus varieties — the Kara, Kinnow and Wilking mandarins and the Trovita orange, *Bull. Univ. Calif.*, 597.
3. Harding, P. L. and Sunday, M. B., 1949, Seasonal changes in Florida tangerines, *Tech. Bull. U.S.D.A.*, **988.**
4. Miller, E. V., Winston, J. R. and Fisher, D. F., 1941, A physiological study of carotenoid pigments and other constituents in the juice of Florida oranges, *Tech. Bull. U.S.D.A.*, **780.**
5. Munsell, H. E., Williams, L. O., Guild, L. P., Troescher, C. B., Nightingale, G. and Harris, R. S., 1949, Composition of food plants of Central America. I. Honduras, *Food Res.*, **14**, 144—164.
6. Munsell, H. E., Williams, L. O., Guild, L. P., Troescher, C. B., Nightingale, G., Kelley, L. T. and Harris, R. S., 1950, Composition of food plants of Central America. IV. El Salvador, *ibid.*, **15**, 263—296.
7. Munsell, H. E., Williams, L. O., Guild, L. P., Troescher, C. B. and Harris, R. S., 1950, Composition of food plants of Central America. V. Nicaragua, *ibid.*, **15**, 355—365.
8. Munsell, H. E., Williams, L. O., Guild, L. P., Kelley, L. T., McNally, A. M. and Harris, R. S., 1950, Composition of food plants of Central America. VI. Costa Rica, *ibid.*, **15**, 379—404.
9. Oppenheim, J. D., 1927, A preliminary note on the origin of the "Jaffa Orange", *Genetica*, **9**, 516—520.
10. Tanaka, T., 1922, A new feature of bud variation in citrus, *Circ. U.S.D.A.* No. 206, 8 pp.
11. Webber, H. J., 1943, Cultivated varieties of citrus, *The Citrus Industry, I. History, botany and breeding*, pp. 475—668, University of California Press, Berkeley, 1028 pp.

AN INVESTIGATION INTO THE PROCESS OF FLOWER AND FRUIT ABSCISSION OF THE SHAMOUTI ORANGE*

A. SHAVIT

Department of Horticulture, Faculty of Agriculture of the Hebrew University, Rehovot

ABSTRACT

(1) Of the Shamouti flowers examined, 77% were found defective.
(2) Staminate flowers in the Shamouti represented 6.2% of the total number of flowers in normal, and 11% in zinc deficient trees.
(3) Two phenomena, appearing in 90% of the cases, indicate in advance the abscission of Shamouti flowers: an intermediate notch and a pronounced colour differentiation of the pedicel.
(4) The seasonal march of abscission is discussed.
(5) The shed flowers were were shed according to the developmental stage reached: in normal trees, 15.6% and in zinc deficient trees 35.8% were shed before opening. 18.8% normal, as against 13.4% zinc deficient, were shed in the stage of well-developed, open flowers, while 6.2% and 11%, respectively, were staminate flowers. The stage of young fruit was reached by 45.1% of the total in the normal, but only by 23.8% in the zinc deficient trees.
(6) At the peak of the blossoming season, the daily loss due to abscission of flowers amounted to 48.58 gm of dry weight. The total loss of a medium Shamouti tree is about 1 kg of dry weight per season.
(7) A medium sized Shamouti tree lost by abscission of flowers 34.61 grams N and 3.16 grams P. Flowers appearing later in the season were found lower in both N and P content than those appearing earlier.
(8) The blossoming process was accompanied by a severe abscission of leaves.

INTRODUCTION

The number of flowers produced by citrus is, generally speaking, vastly in excess of the number of fruits attaining maturity. The many thousands of flowers which bloom during a relatively short period, in addition to the flush of young leafy shoots appearing in spring, probably drain the nutrient reserves of the tree to a considerable degree. However, information in the citricultural literature about this subject is scarce.

In the U.S.A., several authors like Haas (1935, 1947, 1949), Cameron (1934, 1937, 1945), and others, studied problems connected with blossoming and abscission of flowers and fruit. Mainly the chemical composition of the flowers has been investigated.

In the present study we endeavoured to elucidate the relationship between the total number and the morphological character of the shed flowers and fruit on the one hand and the final yield on the other, and to contribute thereby towards the attainment of the following ends:

(1) Establishment of pertinent morphological properties of the citrus flower and of early symptoms of its abscission.

* Abridged summary of a M.Sc. thesis presented to the Faculty of Agriculture of the Hebrew University.

Received March 14, 1956.

(2) Determination of the progress of abscission as a function of time for the entire growing season, and of the relative shares of flowers (shed at various stages of their development), and young and mature fruits.

(3) Assessment of the deviation from the normal course of abscission in zinc deficient trees.

(4) Determination of the quantities of nitrogen and phosphorus lost by the abscission of flowers.

MATERIALS AND METHODS

The observations were carried out in the blossoming seasons of 1953 and 1954 in the citrus groves of the Agricultural Research Station and other groves at Rehovot.

In the first season 100 flowers each of Shamouti and other orange varieties, were tagged. In the second season, 300 Shamouti flowers were marked for observation. The purpose in view was to establish the influence of external factors on abscission and to detect morphological characters appearing before the onset of this process.

Four Shamouti trees, on sweet lime stock, were selected in the grove of the Agricultural Research Station. They had been planted in 1935 on red sandy soil at distances of 3 m in rows 6 m apart. On an average, the trees were 3.20 m high, and their tops measured 3 m across in one, and nearly 4 m in crosswise direction. Rugs were spread under the experimental trees, and all shed material was collected every 2 to 8 days and sorted out into 7 groups, according to stages of flower development. The number of flowers in each group was recorded.

Funnels for the collection of flowers were attached to selected branches of trees showing symptoms of severe zinc deficiency. In this manner, all shed flowers could be collected.

The flowers from each tree collected on each occasion were kept apart and dried to constant weight in an oven at 65—70°C. In view of the fact established by Haas (1935) and Cameron and Appleman (1934) that different parts of flowers vary in the amount of their chemical constituents, an effort was made to collect all parts of each flower including anthers, fllaments and petals that usually shed separately. The dry material was ground in a Wiley mill into a powder and passed through a 40-mesh sieve. Samples of the powder were drawn from every batch of flowers, and their nitrogen content determined by the micro-Kjeldahl method. This enabled us to follow up the changes in the N-content of the shed flowers during the whole flowering period. At the same time, the P-content of the ground flowers was determined by the molybdenum blue method after King.

RESULTS

Causes of abscission of citrus flowers and preceding symptoms

Mechanical damage

Samples of shed flowers collected several times during the blossoming period were examined for symptoms of mechanical damage. Among 273 flowers, 210 (77%) were found injured, the remaining 63 (23%) intact. Injuries were due to: (a) gnawing

or sucking insects attacking various parts of the flower, (b) scratching caused by other parts of the tree (thorns, dry branchlets), (c) fungi developing on the stigma or on the nectaries on the subovarian disk.

Abnormal development of the flowers

A considerable number of flowers were found to be defective. Among all the large-sized flowers shed during two days from a Shamouti tree, 33% showed developmental defects. Of 30 tagged abnormal flowers, none set fruit. The main deviation from the normal in the defective flowers were: misshapen petals — usually very short and therefore not enclosing the inner parts of the flower; pistils shorter and thicker than usual; very short filaments; the pedicel occasionally bent.

Staminate flowers

Staminate flowers may be grouped with other abnormal flowers, but their frequency and unique biological character justify a separate and more detailed discussion. In the Shamouti variety, they are always small (see Figures 1 and 2). At the time of opening, they are only 1—1.5 cm (instead of 2.5 cm) long and only 5 mm (instead of 15 mm) broad. The number of stamens is not increased, but the androecium is longer than in normal flowers (Figure 2), reaching nearly the length of the petals. Instead of the pistil, we find a 1—2 mm long, non-functional rudiment. The staminate flowers are doomed to total abscission; they begin to turn yellow and are shed immediately upon opening, if not earlier. In trees suffering from zinc deficiency, a marked increase of staminate flowers was noted. The average number of such flowers in trees suffering from zinc deficiency reached 11%, while unaffected trees in the same grove averaged 6.2%. We are under the impression that the number of staminate flowers increases with the advance of the growing season, possibly owing to the rising temperature.

Early indications of abscission

On the branchlet (*pedunculus communis*) which forms the axis of the inflorescence, we find small protuberances, or cushions. Here originate the pedicels of the flowers. The pedicels are usually connected to the cushions by a notch (Figure 3, A). In many flowers, a second articulation can be observed a little above the basal notch (Figure 3, B).

The colour of the pedicel is not uniform but, as a rule, passes gradually from pea-green to canary-yellow. However, in many flowers the colour changes abruptly: the base of the pedicel remaining pea-green, and the rest, including the sepals, turning into canary-yellow.

With few exceptions, this abrupt change of colour coincides with the place of the additional notch; it appears at various stages of development and the time elapsing

before it becomes manifest, varies. After formation of a layer of cork and abscission of the flower — including the upper portion of its pedicel — the surface of the apical end of the remaining joint shows convex contours.

It seemed important to ascertain whether there is any causal connection between the formation of an intermediate notch and simultaneous change of colour, on the one hand, and flower abscission on the other. 260 flowers in different development stages were successively tagged for this purpose on trees of different citrus varieties, and were kept under regular observation.

TABLE I

Development of colour differences and intermediate notches on flower pedicels of the Shamouti variety

Abrupt change in colour		Shedding flowers			Fruit-setting flowers		
		Without intermediate notch	With intermediate notch	Total	Without intermediate notch	With intermediate notch	Total
Evident	Number	34	128	162	4	6	10
	%	18.8	71.2	90	5	7.5	12.5
Absent	Number	12	6	18	58	12	70
	%	6.7	3.3	10	72.5	15	87.5
Total	Number	46	134	180	62	18	80
	%	25.5	74.5	100	77.5	22.5	100

We find that pronounced colour differentiation appeared in as much as 90% of the shed flowers, while the remaining 10% of shedding may be assumed to have been due to mechanical injury. This apparently applies also to the 6.7% of flowers without intermediate notches. On the other hand, only 12.5% of the fruit-setting flowers displayed an abrupt difference in colour; among these were cases where the development of an abscission zone proceeded at a slower rate than postfloral growth, and possibly reached its final stage only when the ovary had become a small fruit shedding in due course.

The same explanation is offered for the relatively high percentage (22.5%) of flowers developing intermediate notches, and yet setting fruit.

No intermediate notch was found in 25.5% of shedding flowers. This is an unexpectedly high figure. But since many of these flowers developed a pronounced colour transition at the basal notch, we may assume that in these cases the basal notch coincides morphologically and functionally with the missing intermediate notch.

On the whole, our observations seem to justify the conclusion that a causal connection between the two characters of the pedicel and the shedding of flowers exists in the vast majority of cases.

As a rule, the appearance of a notch and colour differentiation in the pedicel runs parallel with a colour change of the ovary. The ovary of a shedding flower assumes a yellowish tint or else persists in a yellowish-green coloration, while ovaries of setting fruit turn a darker green.

The transformation of the flower into a fruit brings about changes in the manner of abscission. When the ovary reaches a diameter of 8—10 mm, the first abscission takes place at the point of its attachment with the disk, and only later on follows the abscission of the receptacle with disk and sepals from the pedicel. At this stage also occurs a conspicuous thickening of the pedicel, which does not take place in flowers shedding later on.

The seasonal march of abscission

(1) It can be seen in Figure 4 that the abscission of *unopened flowers* lasted 37 days, reaching high figures between the 6th and the 26th day. The peak was reached between the 14th and 16th day, with 7.4% of the total of unopened flowers shedding in the course of 24 hours. Abscission of *open flowers* set in on the 8th day of the abscission period, continuing to its 39th day. A peak (nearly 6% of the total of that group of flowers per day) was reached between the 24th and 27th day. Defining as *fruit-setting flowers* those which show the beginnings of abscission layer between ovary and style, we learn that this group was shed later on and within 25 days only, with a high peak between the 28th and 33rd day (nearly 9% of the total of this group daily). Pooling the three groups we find the peak of the curve of abscission between the 26th and 32nd day, when 4.4% of the total was shed daily.

(2) Figure 5 presents a graphical survey of abscission throughout the season. The columns of the histogram represent totals of the various assortments of shed flowers and fruits, expressed as percentages of the sum total of all shed units, and of fruit which did not shed — separately for the normal and zinc deficient trees.

We find that 15.6% of the flowers that blossomed during the 1953 season on the normal trees were shed *before opening*. Among these were 3.2% "tiny" flowers, i.e., not larger than 1—2 mm, 10.2% small (about half the size of just-opening normal flowers) and 2.2% large (size normal for just-opening, well developed flowers).

25% of the total was shed at the stage of *open flowers*. Of these, 6.2% were staminate in the sense explained before, while the remaining 18.8% were abnormal in other respects, normal or damaged.

14.3% were shed in the stage of *fruit setting flowers*. Altogether 54.9% of the total were shed as flowers before reaching the developmental stage of fruits, during the six weeks between April 2 and May 13.

Abscission of *fruits* from 1 cm in diameter to maturity continued from May 13, 1953 to January 6, 1954, embracing 41.9% of the total. Among these there were only few ripe fruits. The remaining 3.2% reached maturity and could finally be harvested.

(3) The hatched columns of Figure 5 symbolizing the percentages of shed flowers

and fruits from *zinc deficient trees*, differ from those of normal trees in most stages of their development.

In the unopened category, we find 10 (instead of 3) percent tiny flowers, 23.7% (!) (instead of 10%) small flowers, while the figures for large flowers do not differ. A considerable difference exists in the abscission of open *staminate* flowers; it is much higher in the zinc deficient trees, while in other open flowers we find an opposite relationship. In the stage of fruit setting flowers we find 15.6% (instead of 14.3%).

These heavy losses at early stages were responsible for the fact that only 23.8% (as against 45.1%) reached the stage of immature fruit.

Figure 6 shows curves of abscission for normal and zinc-deficient trees. The ordinate indicates daily losses as percentage of the total. We find a gradual rise by steps to the peak of 4.4% in the normal trees followed by a steep decline reaching zero level on the 41st day. In contrast to this, abscission in the zinc deficient trees sets in at once at a relatively high level (mainly unopened flowers), reaching two peaks about the 16th and the 36th days, with a deep depression about the 21st day. The abscission period lasts a few days longer, a fact which can be explained by delayed abscission of well-developed staminate flowers.

(4) Table II gives the total number of flowers counted on each tree and the total dry weight of shedding flowers; the height and horizontal extension of the trees is included to show the relationship between sizes of trees on one hand, number and weight of the flowers on the other. The average total loss of dry matter per tree was 1021 gm.

TABLE II

Sizes of the trees, and data regarding their flowers

Tree No.	Height (m)	Spread of the top		Number of flowers	Dry weight of the flowers (gm)
		Length (m)	Breadth (m)		
1	3.10	2.33	3.80	9201	568.1
2	3.30	3.00	3.80	9750	598.0
3	3.45	3.62	4.25	31418	1870.0
4	3.10	3.00	4.05	19580	1051.8

Figure 7 shows the average daily losses of dry weight in tree No. 4 which most closely approached the average. Between the 14th and the 26th day we find a rather constant daily loss of 43 to 48 gm of dry weight.

The losses of nitrogen and phosphorus

Tree No. 1 lost by abscission of flowers 20.89 gm of nitrogen. The phosphorus loss could not be determined. Tree No. 2 lost 20.04 gm N and 1.43 gm P, tree No. 3, 60.87 and 5.09 gm, and tree No. 4, 36.6 gm and 2.96 gm, respectively. The average loss per tree works out at 34.61 gm N and 3.16 gm P.

Figure 8 shows the average daily loss per tree of both nutrients due to abscission of flowers, throughout the blossoming season. The maximum losses occurred between the 14th and 16th day, when 1.7 gm N and 0.15 gm P were lost every day. Later on, the losses diminish, reaching a level of about 1 gm N and 0.07 gm P towards the end of a month.

The changing composition of shed flowers is given in Figure 9. Both N and P, plotted as percentages of dry substance, show a gradual decline from a high content of about 4.4% N and 0.34% P on the eighth day, to figures lower by about one fourth to one third on the 26th day. For comparison, we have plotted analogous results obtained by Cameron et al. (1934) for N from samples of 20—200 flowers each shedding (or possibly plucked) from a 12 year old Valencia tree in California. A comparison of initial and final figures as given by the American authors with those recorded for our tree No. 3, shows a striking similarity, though intermediate figures differ, being slightly higher in the case of the Californian Valencia tree. Since the American authors did not carry out a complete analysis of blossoming and abscission, the reason for this difference cannot be indicated with certainty. Possibly, it may be sought in a different developmental rhythm of the flowers in the two varieties.

Abnormal leaf abscission

About the beginning of the last week of the blossoming season, a very marked abscission of green leaves set in. It reached rather exceptional proportions, and it probably represents the principal thrust of abscission — or at least one of the major ones — during the year. The shed leaves were of all sizes and contained also very young leaves of the current season, not more than two months old. During the whole blossoming season, instances were also observed of shedding of the apex of young shoots, and this became rather frequent during the last week. This phenomenon has also been observed by Zacharia (1951) and De Beer (1937). The processes involved may be connected with the drain of certain foodstuffs to the flowers from other organs.

DISCUSSION

The abscission of citrus flowers is not, in itself, undesirable, since a full set would lead to the production of an abundance of small fruit of low commercial quality. On the other hand, a thorough knowledge of the processes of abscission and its causes might help towards a more effective use of the productive capacities of the tree. A deeper insight into the causes of abscission might also prove valuable in cases of exaggerated abscission resulting in a drastic reduction of yield, such as often occurs with the Clementine mandarin in Israel and, according to Coit and Hodgson (1919), with the Washington Navel orange in the inner valleys of California.

Though the presence of flowers with abortive vestiges of pistils is rather widespread in the Shamouti variety, we are inclined to consider it not as a normal phenomenon, but rather as a consequence of a subnormal supply of certain nutrients, such as nitrogen or zinc. The flowers compete with each other for the nutrients and the losers are unable

to develop the primordia of their pistils. This impression is strengthened by the fact that staminate flowers become more numerous towards the end of the blossoming season, when the spring flush of vegetative growth and the development of the earlier flowers have drained the nutrient reserves to a considerable extent. Loehwing (1940) maintains that low supply of nitrogen may provoke the formation of male flowers; he cites Combes for the view that mineral nutrients are first directed to the developing stamens and only later on to the pistil.

We have observed that flowers with short, narrow and often supernumerary stamen-like petals, were ultimately shed in all cases, and, as a rule, before reaching the size of normal flowers. While normal flowers are closed in the early stages of their development, these abnormal flowers are open, though their pistils, styles and stamens continue to grow. Coit and Hodgson (1919) also stress the fact that a large proportion of shedding flowers in the Washington Navel variety is shed as a consequence of abnormal morphological characters, of which most important are defective pistils in various stages of atrophy. Our observations on Shamouti point to mechanical damage as a more frequent cause of abscission than abnormal development, though it must be admitted that the latter represents a serious handicap in the competition between flowers and therefore abnormal flowers form a substantial contingent of the total shed material.

Webber (1948) stated that few data were available on the numerical ratio between buds and fruit reaching maturity. He cites only the investigation of Reed (1919) on lemon trees. Reed found that of 4440 marked buds, 294 developed mature fruits, i.e. only 6.62%. Reece (1945) tagged groups of flowers on trees belonging to different orange varieties and subsequently recorded the following percentages of mature fruit: Valencia 4.81%, Hamlin 3.35%, Pineapple 6.65%. Our figures for Shamouti, a variety limited in its seed-bearing capacity, compare favourably with those for Valencia and Hamlin, but fall considerably behind the percentage obtained for Pineapple, known for its abundance of seeds which raises the odds for setting and maturing of fruits.

Of a tree bearing 4201 flowers, 424 ripened: 4.6%
Of a tree bearing 9750 flowers, 520 ripened: 5.33%
Of a tree bearing 19580 flowers, 564 ripened: 2.88%
Of a tree bearing 34418 flowers, 702 ripened: 2.23%

While Zacharia (1951) investigated the effect of the position of flowers in the inflorescence on their chances of development, Haas (1949) devoted his attention to the study of relative potentialities of early and late opening flowers. His results demonstrate that the chances of flowers to develop into mature fruits improved gradually with the progress of the blossoming season. The percentage of maturing fruits was nought with flowers opening on March 19, but 50% on April 22. Haas suggests the following reasons: (1) early opening flowers face greater competition for nutrients, on account of the spring flush of leaves; (b) early setting is adversedly affected by cool temperatures.

Our own observations in Israel stand in marked contrast to those of Haas; it appears

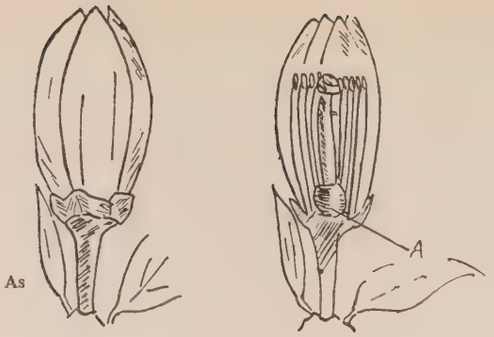


FIGURE 1. (a) A normal flower at its opening stage. (×2). (b) As above, in longitudinal section. A — a normal pistil.

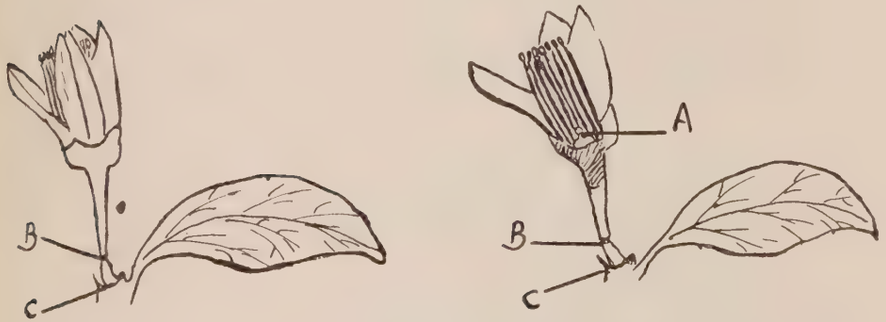


FIGURE 2. (a) A staminate flower at its opening stage. (b) As above, in longitudinal section. A — rudiment of pistil, B — intermediate notch, C — basal joint.

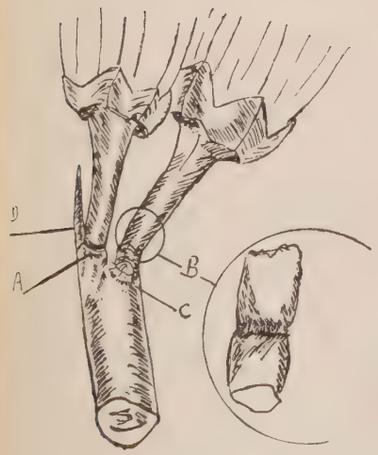
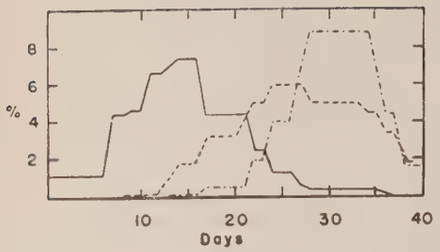
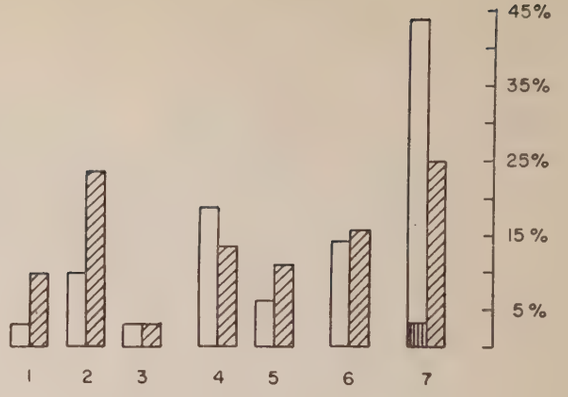


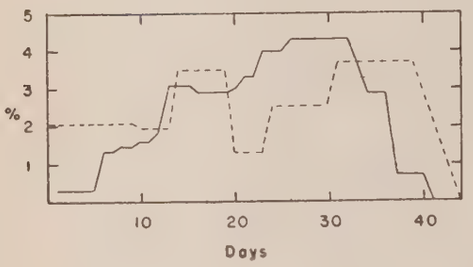
FIGURE 3. Pedicels of flowers of Shamouti orange. A — basal joints, B — intermediate notch, C — small bud in the axil of the flower stalk, D — bract.



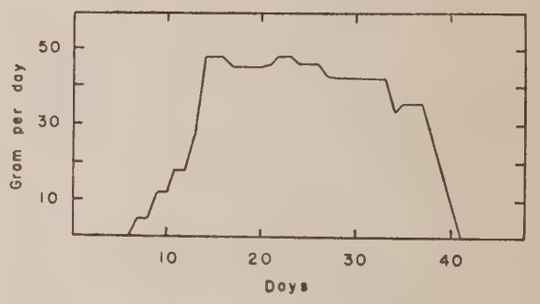
4



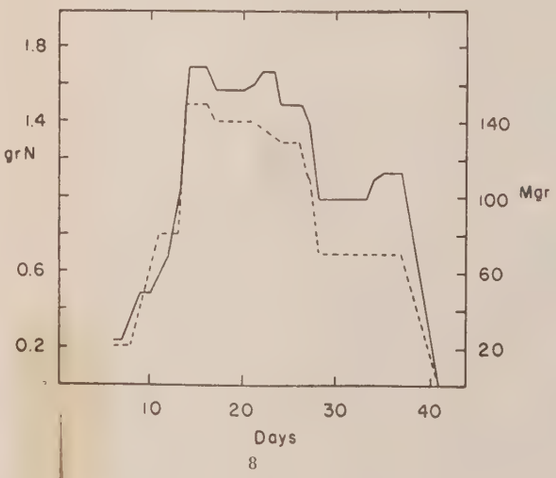
5



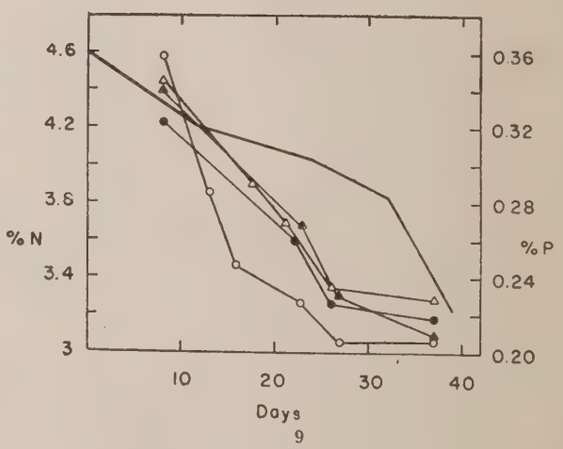
6



7



8



9

Figure 4. Daily abscission of flowers according to stages in development: ———— buds, — — — — — open flowers, — . — . —. fruit setting flowers. Days beginning 2.IV.53.

Figure 5. Total abscission of the different categories of flowers (percentage of the grand total). (1) Tiny buds, (2) medium buds, (3) large buds, (4) hermaphroditic flowers, (5) staminate flowers, (6) fruit setting flowers, (7) fruits. (I eft columns —— normal trees, right columns —— zinc deficient trees. The unhatched portion above the cross bar indicates fruit shed before maturity, the lower hatched portion indicates fruit picked ripe.)

Figure 6. Seasonal march of total abscission of flowers:————normal trees, — — — — — zinc deficient trees.

Figure 7. Total daily losses of dry matter by abscission of flowers.

Figure 8. Daily losses of nitrogen and phosphorus by abscission of flowers (losses of N in gm, of P in mg).

Figure 9. Nitrogen and phosphorus content of shed flowers as percentage of dry matter: ●————● tree No. 2 — nitrogen, Δ————Δ tree No. 2 — phosphorus, O————O tree No. 3 — nitrogen, ▲————▲ tree No. 3 — phosphorus, ———— Valencia tree in California.

that early opening flowers have the better prospects for turning into mature fruits, as they enjoy a better supply of nitrogen and phosphorus and a precedence in the provision of other important nutrients. For the explanation of the antithesis, it should be kept in mind that climatic conditions in Israel differ from those prevailing at blossoming time in California. While in Israel low night temperatures are apparently unimportant as a factor restricting early fruit set, late fruit set is probably more hampered by periods of hot and dry weather, typical of the later part of the blossoming season in this country, and by higher percentages of staminate flowers.

Now passing to the problem of nutrient requirement, it seems important to state that nitrogen and phosphorus are the main and often the only fertilizing materials used by Israeli citrus growers. Our main object was to elucidate the nutritional requirements of the trees during the little-studied period of their annual growth cycle — the blossoming season, when the soil in the grove is often covered by a snow-white layer of shed flowers.

The loss of nutrients involved in flower abscission is the more important, as the production of the flowers and also of the spring leaves must mainly be charged to the account of reserves accumulated in the tissues of the tree. This has been proved by Cameron and Compton (1945), who established a pronounced depletion of nitrogen in the main organs of orange trees which persists throughout the spring, while Oppenheimer (1956) and Monselise (1953) insist upon the fact that the roots of Shamouti trees are in a state of rest and are covered to their tips by cork layers, while growth of the leafy top proceeds in spring. Observations by Cameron (1937) show that in March and in April the accumulation of nitrogen in the flowers and young fruits is slow, in contrast to May and June when a sudden increase takes place in connection with renewed root activity.

Comparison with the nutrient content of other organs clearly bears out the fact that the relative importance of the amounts contained in the reproductive organs is rather great, though rarely appreciated sufficiently. We found scattered, but rather impressive figures on this matter in the citricultural literature.

According to Cameron and Compton (1945), a ten-year old Valencia tree contains 900 gm of nitrogen in its vegetative organs. The data of other authors for the total nitrogen content of the leafy top, the yearly crop and the yearly increment in leaves of citrus trees range within the limits of a few hundred grams. Thus Wallace et al. (1951) indicate 625 gm for all the needs of a Valencia tree except for enlarging its branch system. According to Cameron and Compton (1945), 228 gm are needed for the yield of a Valencia tree, while 100 gm have to be allowed for the skeleton growth of a tree of the same variety. Monselise and Heymann-Herschberg (1953) arrive at 229.7 gm as the nitrogen content in all the leaves of a Shamouti tree. In contrast to this, the loss due to shed organs is of the order of only tens of grams. Cameron (1937) found the total shed immature fruit of an unusually strong June drop to contain 49.7 gm N, a figure comparing reasonably well with the 60.87 gm established by us as the average of the four Shamouti trees examined.

The gradual decline in nitrogen content of the flowers established in the present study is matched by a similar trend found in Valencia trees in California. Our view that it is due to an increasing shortage of nitrogen in the tree, is in line with the findings of Monselise and Heymann-Herschberg (1953). These authors showed that the N-content, per surface unit, of young Shamouti leaves is higher than that of mature leaves, except in May. Moreover, the N-content of the young leaves was higher in August than in May, and this can be considered as a further proof that the flowers compete with the other organs of the trees.

The decline in N-content of late-appearing flowers is by no means a mere consequence of accumulation of dry matter. Cameron et al. (1934) analysed flowers of the same weight plucked from one and the same tree early and late in the season and found a downward trend in the nitrogen content. In the light of all the cited observations, one is led to the conclusion that the shortage of nitrogen during and shortly after blossoming time constitutes the main factor limiting the setting of fruit.

Possibly shortage of nitrogen is also a contributing factor in the unfruitfulness of Clementine, since certain types of this variety form enormous numbers of flowers. Oppenheimer (1948), who studied this problem thoroughly, paid little attention to its nutritional aspect.

In order to overcome the temporary shortage of nitrogen, it would seem appropriate to spray compounds like urea on the leaves, instead of fertilizing the soil at a time when absorption by the roots is impeded. Accordingly, Dr. Monselise with the help of the present author undertook a first experiment with N-sprays on Clementines in 1954, but failed to obtain a higher fruit set.

Shortage of phosphoric acid is probably less important than shortage of nitrogen in the abscission of citrus flowers and fruits. While phosphorus deficiency is rarely observed in the field, this nutrient easily migrates from the mature to the young organs, and especially from mature leaves to the blossoms (Smith and Reuther 1950). Accordingly, Heymann-Herschberg (1950) found a sudden decrease of P during the spring flush in the mature leaves of the Shamouti.

Studying the effects of different nitrogen concentrations and of dates, Haas (1947) found a decrease in the phosphorus content of the flowers during the blossoming period at all concentrations of nitrogen. These findings are in line with ours.

Regarding the phosphorus requirements of the flowers in relation to the total content of the trees, there are a number of indications in the horticultural literature. Wallace et al. (1951) found a P content of 11.4 gm in the shed flowers and fruits of a ten-year old Valencia tree, while the total yearly consumption was 68.7 gm. Turning to the Shamouti orange, Heymann-Herschberg and Monselise (1953) found in the foliage of a tree with 25,603 young leaves not more than 7.5 gm phosphorus. In comparison with this figure, the average of 3.01 gm of P shedding from one tree in our experiment with shedding flowers, is not negligible.

REFERENCES

1. Cameron, S. H. and Appleman, D., 1934, Total nitrogen in developing flowers and young fruits of Valencia orange, *Proc. Amer. Soc. hort. Sci.*, **32**, 204.
2. Cameron, S. H., 1937, Loss of nitrogen through abscission of immature oranges, *Proc. Amer. Soc. hort. Sci.*, **34**, 88.
3. Cameron, S. H., and Compton, O. C., 1945, Nitrogen in bearing orange trees, *Proc. Amer. Soc., hort. Sci.*, **46**, 60.
4. Coit, J. E. and Hodgson, R. W., 1919, An investigation of the abnormal shedding of young fruit of Washington Navel orange, *Calif. Univ. Publ. agric. Sci.*, **3**, 283.
5. De Beer-Wallenstein, 1937, Periodicity of the formation of shoots and buds in citrus, *Palest. J. Bot. hort. Sci.*, **1** (3), 5.
6. Haas, A. R. C., 1935, Chemical composition in flowers of citrus varieties, *Proc. Amer. Soc. hort. Sci.*, **33**, 61.
7. Haas, A.R.C., 1947, Effect of fertilizer and rootstock on total phosphorus content of citrus flowers, *Soil Sci.*, **41**, 239.
8. Haas, A. R. C., 1949, Orange fruiting in relation to the blossom opening period, *Plant Phys.*, **24**, 481.
9. Heymann-Herschberg, L., 1950, Soil and leaf analysis as indicators of fertilizer requirements of Shamouti orange groves, *Ktavim, Agric. Res. Sta., Rehovot*, **1**, 25.
10. Loehwing, N. F., 1940, Mineral nutrients in relation to flower development, *Science*, **92**, 517.
11. Monselise, S. P., 1953, The growth of citrus roots and shoots under different cultural conditions, *Palest. J. Bot.Rehovot*, **6**, 43.
12. Monselise, S. P. and Heymann-Herschberg, L., 1953, Influence of exposure and age on dry matter content, area and mineral composition of Shamouti orange leaves, *Proc. Amer. Soc. hort. Sci.*, **62**, 67.
13. Oppenheimer, H. R., 1948, Experiments with unfruitful "Clementine" mandarins in Palestine, *Bull. Agric. Res. Sta., Rehovot*, No. 48.
14. Oppenheimer, H. R., 1956, *Cultivation of Citrus Trees* (Hebrew), Hassadeh Library, Tel Aviv.
15. Reece, P. C., 1945, Flowering habit and orange fruit-set, *Proc. Amer. Soc. hort. Sci.*, **46**, 81.
16. Reed, H. S., 1919, Certain relationships between the flowers and fruit of the lemon, *J. agric. Res.*, **17**, 153.
17. Smith, A. and Reuther, W., 1950, Seasonal changes in Valencia orange trees, *Proc. Amer. Soc. hort. Sci.*, **55**, 61.
18. Wallace, A. W., Cameron, S. H. and Muller, R. T., 1951, Seasonal use and loss of nutrients and dry matter in orange trees, *Proc. Amer. Soc. hort. Sci.*, **58**, 4.
19. Webber, H. J. et al., 1948, *Citrus Industry*, Vol. **2**, University of California Press.
20. Wilson, Robert F., 1938, *Horticultural Colour Chart*, Publ. Brit. Colour Counc.
21. Zacharia, D. B., 1951, Flowering and fruit-setting of shamouti orange trees, *Palest. J. Bot. Rehovot*, **8**, 84.

STUDIES ON THE VIABILITY OF CITRUS SEEDS AND CERTAIN PROPERTIES OF THEIR COATS*

AHARON COHEN

*Faculty of Agriculture of the Hebrew University, Rehovot***

ABSTRACT

A method for testing the viability of citrus seeds by means of resazurin is described.

It is little time consuming (54 hours) as compared with the several to many weeks required by germination tests, and allows a fair evaluation of seed quality for the use of nurserymen.

The action of seed coats in delaying germination has been investigated at 25°C. It has been found that removal of the coats accelerates germination; removal of both testa and tegmen is significantly more efficient than removal of the testa only.

Intact seed coats delay water intake for several days. They seem to reduce the carbon dioxide output of seeds. Water extracts of the testa contain a substance inhibiting germination and growth of wheat grains and citrus seeds. This inhibiting factor is removed or inactivated by stratification during two weeks.

Since treatments of coats, effective in speeding up the germination of certain other seeds, did not promote the germination of citrus seeds under nursery conditions, it is believed that the low temperature is the main factor responsible for delaying citrus germination during the sowing season.

INTRODUCTION

Several investigators have emphasized the fact that citrus seeds lose their capacity to germinate very rapidly if stored under dry conditions (Barton 1943, Elze 1949, Richards 1952).

Since the time elapsing between sowing and germination of citrus seeds is rather long, their initial germinability may moreover decrease due to adverse edaphic conditions. Any test based on counts of germinating seeds will yield results of dubious value for at least two reasons:

(a) Environmental conditions during the test are, as a rule, different from those obtaining in the nursery; the sowing season may pass before it has been possible to assess the germination percentage of the seeds.

(b) The bulk of the seeds may be damaged owing to long storage by the time the results of the germination test have become available.

It is essential, therefore, that the germination percentage of citrus seed be determined more rapidly than by counts of germinating seeds. A suitable biochemical test would seem appropriate for this purpose.

* English summary of a M.Sc. thesis accepted by the Faculty of Agriculture of the Hebrew University in 1955.

** Present address: Agricultural Research Station, Rehovot.

Received March 14, 1956.

Polyembryony of citrus seeds renders it even more difficult to assess both actual germination percentage and viability of seeds. The several seedlings which frequently arise from a single seed, cannot be considered as equivalent to each other or to solitary seedlings; their capacity for survival and usefulness as nursery stocks may indeed be unequal.

Since germination time can be effectively reduced by removing the seed coats, at least at 25°C (Monselise 1953), it may be assumed that the coats exercise some kind of inhibition of physical or chemical nature.

The present work was undertaken in order to develop a reliable test of viability and to elucidate some of the problems outlined above.

VIABILITY TESTS

Viability of seeds has been tested by various biochemical stains which often indicate the activity of dehydrogenases. Most indicators, such as sodium selenite or 2,3,5-triphenyl-tetrazolium chloride (Flemion and Poole 1948), are poisonous to seeds, and the stain reactions cannot therefore be tested against actual germination.

A substance harmless to seeds has been recently proposed by Plaut and Halfon (1953). It is *resazurin* (blue at *p*H above 6.8, red at *p*H below 5.3), which readily gives off its oxygen and is thus (irreversibly) reduced to *resorufin* (red at *p*H above 6.4, orange at *p*H below 4.8). Resorufin can be further reduced to colourless *dihydroresorufin*, this reaction being reversible. Since the colours of these substances differ, the colour retained by seeds or parts of seeds can be used to evaluate the amount of reduction and thus provide a measure of viability. Since both resazurin and resorufin assume different colours at different *p*H levels, the *p*H at the surface of tissues tested plays an important part in the reaction.

Resazurin is used in the dairy industry to test the degree of infestation of milk by microorganisms. It has recently been used successfully to test seed germinability of peas, beans, cucumbers (Plaut and Halfon 1953) and cereals (Plaut and Heller-Cohen 1955). In the case of the vegetable seeds mentioned, it has been found necessary to strip off the testa.

In preliminary experiments, large quantities of sour orange seeds were stored indoors, in dry sand, during the winter months and samples were tested at weekly intervals. On each occasion, one sample of seeds was stripped (both seed coats) and treated with resazurin, another was stripped and left untreated, and a third was left intact and untreated. All the samples were germinated at 25°C, and an additional sample of intact seeds was sown at the same time in a cold greenhouse.

The treatment of the first mentioned sample was as follows: after removal of the coats, the seeds were soaked for 24 hours in a 50 p.p.m. solution of sodium resazurate, and then transferred onto moist filter paper in plastic dishes, kept for 24 hours at 25°C in a germinator, and finally classified according to colour of root tips and returned to the germinator till germination was complete. When first removed from the resazurin solution, seeds were colourless to faintly pink, but after a few minutes of contact

with air, they assumed deeper tints, probably as a consequence of partial reoxidation of dihydroresorufin to resorufin. While the cotyledons of most seeds were faintly pink after 24 hours, red root tips were found only in seeds of low viability. The final germination of seeds containing embryos with colourless root-tips averaged 86 percent, while that of seeds with coloured root tips did not exceed 12.5 percent.

The following conclusions can be drawn from the above tests:

Treatments with resazurin at the concentration used did not reduce the germination percentage of seeds as compared with untreated controls. This fact has already been indicated by Plaut and Halfon (1953) for other seeds.

Significant differences in ultimate germination percentage were found between seeds kept in the germinator and others sown in the soil, the latter attaining a much lower percentage. This confirms the results obtained by Monselise (1953). The largest differences were found after prolonged dry storage (six weeks): stripped seeds in germinator maintained at constant temperature of 25°C, gave final germination of 83 percent, as against 48 percent recorded for intact seeds in the soil.

Resazurin may be used to measure the germinability of citrus seeds, but the method requires improvement along two lines: (a) finding a way to test intact seeds, since the stripping of the coats is very tedious, and in view of the fact that the germination percentage of stripped seeds is higher than that of intact seeds; (b) working out a staining treatment which would provide as wide a range of colour reaction as possible.

In the following account, a step by step description is given of the method as so far developed. The reasons underlying each stage of the procedure are expounded, although the experiments which led to its development cannot be described in detail. This method is suitable for sour orange seeds, and should probably apply as well to other citrus species with, perhaps, minor modifications.

(1) Intact seeds are soaked in tap water for 24 hours, at about 25°C. It was shown that longer soaking impairs germination. Seeds absorb in 24 hours 50 to 90% of the total amount of water absorbed in 60 hours.

(2) Acidity of water and seed coats is slowly neutralized by means of N/10 NaOH, using resazurin as an indicator and stirring thoroughly. This is necessary since the slightly acid reaction of seed coats may result in similar colours with both resazurin and resorufin; moreover, the widest range of colours is obtained near neutrality.

(3) Seeds are dipped in a fungicide, e.g. 1% Caspan improved, for one minute, in order to prevent growth of microorganisms that may influence staining by their enzymatic activity. In many cases non-viable intact seeds, if not disinfected, assumed tints similar to those of viable seeds, as a result of metabolic activity of microorganisms developing on their coats. Analogous observations were made on stripped seeds. Microorganisms are known to attack mainly seeds of low viability (Gadd 1943).

(4) Intact seeds are soaked for 6 hours in a solution of 100 p.p.m. sodium resazurate in water. 1 ml of the solution per seed appears to be adequate. For tests with stripped seeds, a concentration of 50 p.p.m. was found sufficient.

(5) Seeds are drained free from solution, spread on moist filter paper, and kept at 25°C for 24 hours. The number of seeds in each colour group is then noted.

Different concentrations and periods of soaking and colouring were tested extensively with both stripped and intact seeds. It was found that, with a concentration of 150 or 100 p.p.m., the most satisfactory colour differentiation took place upon exposure on moist filter paper for 24 hours, after soaking in resazurin for 6 or 12 hours. The shorter period of soaking is preferable for the following reasons: (a) it reduces the duration of the test; (b) the degree of reduction of the resazurin solution is a function of the duration of seed immersion. Thus, the shorter the period of soaking the smaller are the differences in colour appearing upon exposure of the soaked seeds to air.

(6) The seeds are finally matched against previously prepared standards or against a colour chart. Four different colour groups may be distinguished (sometimes intermediate hues may also be detected), as shown in Table I.

TABLE I

Colour of intact sour orange seeds treated with resazurin, and corresponding germination percentages

Group No.	Range of colours	Viability	Germination percentage
0	blue to violet blue	none	0
I	purple to reddish purple	low	15—30
II	purplish red to red	fair	40—60
III	reddish pink to pink	high	80 and more

The above percentages of germination have been established by checking results with germination in soil. In one particular trial we found: in group 0, no germination; group I, $18 \pm 5.6\%$; group II, $50 \pm 3.8\%$; group III, $80 \pm 7.6\%$. Using the above method and standards, patches of seeds of unknown germination percentage were tested. The batches were obtained by mixing seeds which had been subjected to different periods of heating. The results of tests carried out in five replications on one of these mixtures are reported in Table I and in Figure 1.

TABLE II

Comparison of predicted and actual germination percentage of sour orange seeds stained with resazurin and subsequently sown in the soil (mixed seed samples of varying viability)

No. of sample	1	2	3	4	5
Predicted germination*	31	31	33	33	32
Actual germination	29	32	31	31	32

* Within the range of each colour group, intermediate hues were distinguished, and these were allotted intermediate numerical assignations, with the aid of the colour chart.

It is evident that a rather high degree of accuracy in predicting germination percentage has been achieved, even though the samples were of relatively low germinating capacity and could therefore be considered as difficult material for assessment by staining tests.

Preliminary experiments were also undertaken with sweet lime, rough lemon and Cleopatra seeds. The technique worked out for sour orange was employed, except that the interval between removing the seeds from solution and assessment of colour was cut down to 18 hours. The responses of sweet lime seeds were attributed to two colour groups only: blue and purple, corresponding to 18.2±9.0 and 82.0±4.1 percent germination, respectively. Staining reactions were also obtained with rough lemon and Cleopatra seeds, but further experimentation is needed before their interpretation can be attempted.

FACTORS AFFECTING THE LENGTH OF THE GERMINATION PERIOD

Influence of seed coats

Four batches of fresh sour orange seeds (a) untreated, (b) intact, soaked in water changed three times at 30°C for 24 hours, (c) with testa removed, (d) with both testa and tegmen removed, were germinated at 25°C.

The length of the germination period was expressed in two ways:

(a) the number of days required for the germination of 50 percent of the ultimately germinating seeds;

(b) average germination period in days $T = \frac{n - t_n}{N}$

where t_n = period in days elapsing before the germination of each seed;

n = number of seeds germinated at each t_n;

N = total number of seeds germinating.

Table III shows the results obtained with 1000 sour orange seeds (5 replicates of 50 seeds for each treatment).

TABLE III

The length of the germination period of sour orange seeds as influenced by different treatments (at 25°C)

	Untreated control	Intact, soaked in tap water	Testa removed	Both seed coats removed
Number of days required for 50% germination	28	23	13	3
Average germination period in days	32	27	17	6

The differences between all the treatments are highly significant. The seed coats constitute a definite hindrance to germination under these conditions. Soaking hastens germination as compared with unsoaked controls, this being possibly due to a cumulative effect of temperature, water absorption and washing out of inhibitors.

Chemical inhibition

Intact seeds after thorough washing (a), seeds from which the testa had been removed (b), seeds from which both testa and tegmen had been removed (c), seed coats alone (d), were soaked in distilled water for 24 hours, at 25°C.

5 ml of liquid from the soaking beakers, each containing a water extract from 5 sour orange seeds, were poured into Petri dishes containing 50 wheat kernels (*Triticum durum*, Merarit variety) on dry filter paper.

Germination and development of roots have been evaluated after 68—70 hours at 18°C by means of the following grading scale: 0 = no germination; 1 = one root, not more than 5—6 mm long; 2 = up to three roots, with cumulative length not exceeding 15 mm; 3 = three roots, of which at least one measures 15 mm or more. The *index of germination*, which is here defined as the product of grades and the number of rated seeds, has been employed for comparison with germination in water. Figures 2 and 3 show the inhibition of wheat germination produced by extracts from different parts of citrus seeds and by seeds stored in different ways. Significance of inhibition over control at $P = 0.05$ (*) and 0.01 (**) was assessed by individual degrees of freedom.

These data suggest that an inhibitor is extracted from intact seeds and especially from the testa by the water used for soaking. Even a short period of stratification seems to inactivate this inhibitor very markedly. While a positive correlation ($r = +0.681$**) was established between the inhibition of germination and the total acidity of the solution, no significant correlation could be detected between the inhibiting action of the solution and its *p*H.

In addition to the experiments with wheat, the effect of extracts from fresh, thoroughly rinsed sour orange seeds on stripped seeds of the same species has also been investigated. In this case, the extent of inhibition ranged from 15 to 30 percent. Dilute citric acid of the same total acidity as the seed extract, but with a lower *p*H, induced stronger inhibition.

Influence of seed coats on respiration

The output of carbon dioxide in the respiration of sour orange seeds was determined by a conductometric method using N/10 NaOH as absorbing medium. Table IV shows the results of the determinations which were carried out 12 hours after the interruption of soaking or the stripping of seeds. More carbon dioxide is given off by treated seeds than by controls.

TABLE IV

Carbon dioxide output of fresh orange seeds as affected by different treatments

Treatment	Absolute output (mg CO_2/100 seeds in 24 hours (25°C))*	Relative output	Number of samples
Untreated control	49± 6.5	100	8
Presoaked in water (24 hours, 25°C)	76±10	155	8
Testa removed	103±25	210	4
Both seed coats removed	140± 9.5	286	8

* 100 seeds weigh 21—24 gm.

Influence of injury to seed coats on water absorption

Both the amount of water absorbed and the rate of absorption seem to rise when the seed coats are split open, at least during the first two days of soaking (Table V). When experiments were extended over three days, differences tended to level out.

TABLE V

Water absorbed by intact sour orange seeds and seeds with split coats (water as percent of dry weight)

Hours of immersion	Intact seeds 24	Intact seeds 60	Seeds with split coats 24	Seeds with split coats 60
Initial moisture				
76	87	100	107	108
23	80	95	103	105
5	37.5	82	66.5	92

Presowing seed treatments

Seed treatments by (a) NaOH, N/10 and concentrated (for periods ranging between 20″ and 20′); (b) conc. H_2SO_4 (between 20″ and 60″); (c) soaking in water at 25°C for 24 hours; (d) washing seeds with warm (50°C) running water (about 10 cc per seed); (e) combined warm water and NaOH treatment; (f) soaking in 0.01 p.p.m. 2,4-D for 20 to 24 hours; all failed to induce more rapid germination than in controls, when tried out under nursery conditions and in different seasons. Seeds treated with concentrated sulphuric acid were killed in every case. As a consequence of treatment with concentrated NaOH for 20 minutes the inner seed coat (tegmen) could be peeled off in two separate layers: the outer brownish-gray and brittle, the inner colourless, bright and somewhat elastic. The inner layer represents probably what remains of the endosperm and the nucellar tissues (Webber and Batchelor 1943).

DISCUSSION AND CONCLUSIONS

The method for testing the viability of citrus seeds which has been developed, has certain advantages over other methods. It is fairly rapid (about 54 hours) as compared with germination tests. As against viability tests previously devised (Monselise 1953), much time is saved, since seed coats are not removed. The treatment of seeds as individual units eliminates guesswork concerning selection of an embryo for viability test in polyembryonic seeds. Since the seeds are not killed by the dye, they can be sown after the test for further examination.

It is not claimed that the germination percentage is accurately determined. For reasons already discussed at the beginning of this paper, it is very doubtful whether a valid determination of germination percentages can be made at all in the case of citrus seeds. However, the resazurin method makes it possible to test the quality of seeds and to make a reasonably reliable forecast as to the chances of securing a satisfactory stand of seedlings. The individual examination of seeds allows moreover to decide whether

a given sample is uniform or whether batches of seeds of different degrees of viability have been mixed — a condition which can easily be recognized by an unusual diversity of colours. Such knowledge is not without practical importance.

It can also be assumed that a test carried out with intact seeds provides a better estimate of the viability of the whole seed than colour reactions of root tips (Monselise 1953, Plaut and Halfon 1953) which represent in citrus only a small fraction of the whole seed. Seeds showing stain reactions indicative of low viability may also be more susceptible to disease attack, as shown for peas by Gadd (1943). The number of embryos, their relative size and viability, are undoubtedly of great importance in connection with assessment of seed quality.

Seed coats seem to delay germination which is enhanced by temperatures near optimum. The seed coats act in manifold ways, by hindering water intake, by lowering carbon dioxide output, and — as could be shown at least for the testa — also by chemical inhibition. However, these effects seem to be of rather short duration, e.g. water intake is delayed for only a few days, while chemical inhibition is removed by two weeks' stratification.

No attempt has been made to identify the inhibiting factor found in the outer seed coat. Citrus juice contains an inhibitor which seems to be citric and malic acids (Tetjurow 1938, as cited by Evenari 1949). Prill et al. (1949) have noted some inhibition of wheat growth by the outer seed coat, especially in sour orange seeds where the outer layer is of a mucilaginous character.

The mostly low temperatures which prevail during germination in the covered seed beds in winter, as well as in open seed beds in early spring, seem to be the paramount factor in delaying germination. In our tests, the average germination period of untreated seeds was 54 ± 4 days in January as against 24 ± 1 days in June.

The paramount importance of temperature would appear to be further confirmed by the fact that treatments generally found useful for softening the coats of other slowly germinating seeds had no effect on germination of citrus seeds.

ACKNOWLEDGMENT

The author is indebted to his teachers Dr. S. P. Monselise and Prof. M. Plaut, who suggested this study and helped in its execution. The present English summary was edited by Dr. Monselise and Prof. H. R. Oppenheimer.

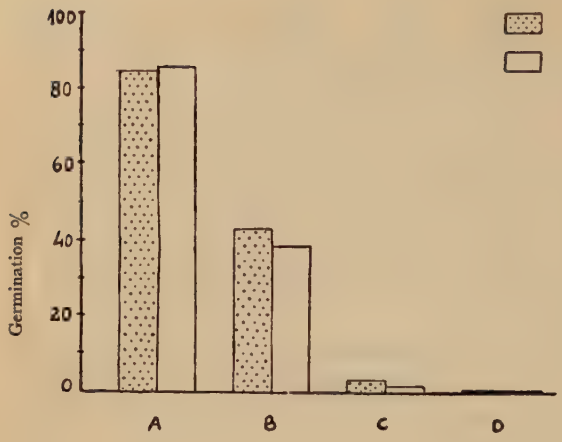


FIGURE 1. Comparison of predicted* and actual germination percentage in four batches of sour orange seeds of different viability stained with resazurin and subsequently sown in the soil (samples of uniform viability).

Staining of seeds

A — Reddish pink to pink
B — Purplish red to red
C — Purple to reddish purple
D — Blue to violet blue

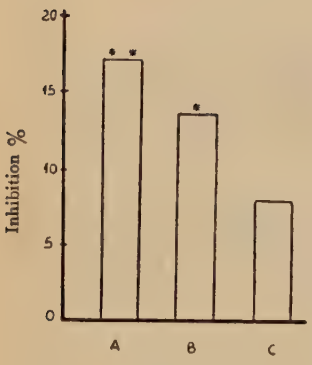


FIGURE 2. Inhibition of germination and growth of wheat grains by extracts from sour orange seeds stored under different conditions (percentages of inhibition have been calculated by deduction of germination indices from 100 = germination index in water).

A — Extract from fresh seeds.
B — Extract from seeds kept in dry storage for two months.
C — Extract from seeds kept stratified in sand for two weeks.

* Significant over control at P=0.05
** Significant over control at P=0.01

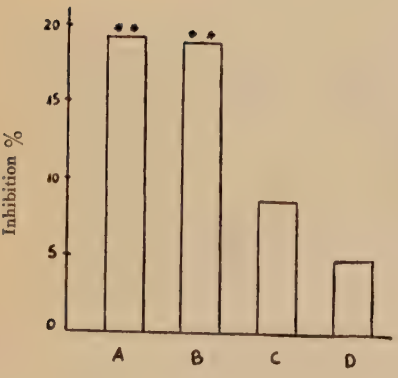


FIGURE 3. Inhibition of germination and growth of wheat grains by extracts of different portions of fresh sour orange seeds (percentages of inhibition have been calculated by deduction of germination indices from 100=germination index in water).

A — Extract from intact seeds.
B — Extract from both seed coats.
C — Extract from seeds without testa.
D — Extract from seeds without testa and tegmen.

** Significant over control at P=0.01

REFERENCES

1. Barton, L. V., 1943, The storage of citrus seeds, *Contr. Boyce Thompson Inst.*, **13**, 47.
2. Elze, D. L., 1949, Germination of citrus seeds in relation to certain nursery practices, *Palest. J. Bot. Rehovot*, **7**, 69.
3. Evenari, M., 1949, Germination inhibitors, *Bot. Rev.*, **15**, 153.
4. Flemion, F. and Poole, H., 1948, Seed viability tests with 2,3,5-triphenyl-tetrazolium chloride, *Contr. Boyce Thompson Inst.*, **15**, 243.
5. Gadd, I., 1943, Vital colouring of pea seeds by means of malachite green, *Proc. Int. Seed Test. Ass.*, **13**, 3.
6. Monselise, S. P., 1953, Viability tests with citrus seeds, *Palest. J. Bot. Rehovot*, **8**, 152.
7. Plaut, M. and Halfon, A., 1953, The viability test of pea, bean and cucumber seeds by resazurin staining, *Proc. Int. Seed Test. Ass.*, **19**, 14.
8. Plaut, M. and Heller-Cohen, O., 1955, New methods of viability testing of seeds, *Hassadeh*, **39**, 87 (Hebrew).
9. Prill, E. L., Barton, L. V. and Solt, M. L., 1949, Effect of some organic acids on the growth of wheat roots in solutions, *Contr. Boyce Thompson Inst.*, **15**, 429.
10. Richards, A. V., 1952, Viability of citrus seeds, *Trop. Agriculturist*, **108**, 186.
11. Webber, H. J. and Batchelor, L. D., 1943, *The Citrus Industry*, vol. I, California University Press.

TOXIC INFLUENCES OF SODIUM AND SULPHATE IONS ON CITRUS SEEDLINGS

P. ZUSMAN

Faculty of Agriculture of the Hebrew University, Rehovot

ABSTRACT

(1) Sweet lime and sour orange seedlings grown in soil supplied with increasing amounts of sulphate ions, were poisoned if the concentration of SO_4^{--} exceeded 1000 p.p.m. With this critical concentration, about 1.05% SO_3 in the dry susbtance of the leaves was found in sour orange, and about 0.9% in sweet lime.

(2) With increasing concentration of sulphate in the soil, the absorption of calcium by the plants fell off.

(3) Sweet lime seedlings grown in soil containing 1.1 milliequivalent of Na^+ per 100 gm soil (exchangeable sodium percentage $\approx$ 22%) developed typical symptoms of injury, while sour orange seedlings remained healthy under the same conditions.

(4) Development of the seedlings was positively correlated with the ratio Ca/Na in their leaves.

INTRODUCTION

It is well known that citrus trees are injured by high concentrations of soluble salts in the soil, and authors like Kelley, Thomas, Loughridge and Hilgard showed at the beginning of the present century that citrus trees growing in saline soils evince characteristic symptoms of injury. Striking symptoms are a yellow discoloration of the leaves at their margins, and a "burn" at their tips, accompanied by stunted growth. Higher concentrations produce leaf fall, dying of twigs, and ultimately the general decline of the trees. It is also known that different citrus species are affected to different degrees, and that species of the lemon group show the highest degree of sensitivity.

High concentrations of soluble salts are found, as a rule, in soils producing salinity symptoms in citrus plants. Among the anions appear Cl^-, SO_4^{--}, HCO_3^-; among the cations Na^+ predominates, but in certain cas eshigh concentrations of Ca^{++} and other cations have also been detected.

Haas and Thomas (1928) described specific toxic symptoms produced by high sulphate concentration. Eaton (1942) showed that lemon trees are less sensitive to SO_4^{--} than to Cl^- ions. High sulphate concentration hinders the absorption of calcium, as shown by Eaton (1942) and Hayward et al. (1946).

In recent years, the attention of students has been attracted by specific toxicity symptoms produced by sodium. Bower and Wadleigh (1949), experimenting with

Received April 3, 1956.

amberlite, a synthetic product used for cation and anion exchange, were able to show that a rise in sodium concentration in the adsorbing complex beyond a certain limit was injurious to plants. This condition was often a hindrance in the absorption of other ions, and especially calcium, by the plants.

Martin et al. (1952) established a considerable reduction in the growth of sweet and sour orange in soils containing more than 14% exchangeable Na^+, while Haas and Brusca (1954), experimenting with seedlings of tangelo, sour orange, Cleopatra mandarin, etc., found a favourable influence of sodium ions on growth with increasing concentrations of Na^+ up to about 250 p.p.m.

In 1955, the present author undertook a study with seedlings of Palestinian sweet lime and sour orange, in order to investigate the influence of soil reaction upon their growth and absorption of nutrients. In the course of this investigation, it was found that the plants were adversely affected by sodium and sulphate ions. The symptoms of injury were specific for each ion and were connected with characteristic changes in the mineral composition of the plants.

MATERIAL AND METHODS

Seedlings were grown from seeds collected from one tree of each of the varieties studied. From these, sturdy and uniform specimens were selected for experimentation; these were two months old when the experiment began.

The seedlings were planted in earthenware pots which were 25 cm broad and 35 cm deep. The pots were painted with a double layer of asphalt inside and outside. An additional outside coat of aluminium paint was meant to reduce heating by the sun; inside, the pots had a third and fourth coat with acid- and alkali resistant varnish. They were then filled with very light brownish-red sand typical of the orange groves of the Jaffa district (moisture equivalent 6.4%; hygroscopic water 0.42%; exchange capacity 5 milliequivalents per 100 gm; *p*H 6.7—7.1).

The soil was fertilized with K, P, N in the customary concentrations, with the addition of Mg, Mn and B*. Aliquots of the soil were treated with sulphuric acid or sodium carbonate and titration curves of *p*H were obtained. The quantities of acid or soda to be added to obtain the desired concentrations of H^+ ions could thus be calculated. Since the *p*H was meant to be the only independent variable, sodium concentrations were adjusted in all treatments to the maximum resulting from the addition of sodium carbonate to the most alkaline treatment. This meant the uniform supply of 260 mg Na per kg dry soil, administered as sulphate alone or in addition to carbonate. It was hoped that this concentration of Na would produce no injury, representing as it did not more than 1.1 milliequivalent per 100 gm soil. The resulting conditions in the pots are shown in Table I.

* To 1 kg dry soil the following amounts were added: 0.01645 kg P_2O_5 as $(NH_4)_3PO_4$; 0.02470 kg N as KNO_3, $Ca(NO_3)_2$ and $(NH_4)_3PO_4$; 0.01833 kg K as KNO_3; 0.00940 kg Ca as $Ca(NO_3)_2$; 0.000620 kg Fe as $FeSO_4$; 0.000036 kg Mn as $MnSO_4$; 0.000018 kg B as H_3BO_3; 0.00940 kg Mg as $MgSO_4$.

TABLE I

Ion concentrations, conductivity and pH in the soil (calculated per weight units of soil dried at 105°C)

Treatment No.	Sour orange					Sweet lime				
	I	II	III	IV	V	I	II	III	IV	V
Na^+ (p.p.m.)	259	259	260	260	260	259	259	260	260	260
SO_4^{--} (p.p.m.)	2293	1410	540	381	—	2293	1410	540	381	—
CO_3^{--} (p.p.m.)	—	—	—	102	339	—	—	—	102	339
Conductivity of initial extract (mmho/cm, 25°C)	6.5	5.3	4.1	3.9	3.4	6.5	5.3	4.1	3.9	3.4
*p*H	4.16	5.53	6.94	7.88	8.88	4.40	5.38	7.09	7.88	9.02

Specific symptoms of damage appeared on the sweet lime seedlings grown with high concentrations of sulphate as early as 8 days after planting. The periphery of the leaves turned yellow, and the discoloration spread towards the central vein, penetrating between its lateral ramifications. Later on, the tips of the affected leaves and their margins turned brown, as a consequence of necrosis of the affected areas (see Figure 4, left). Finally, leaf abscissions, mainly of the mature leaves, followed. The symptoms agree with those described by Haas and Thomas (1928) as characteristic for excess of sulphate. Ten days after the appearance of the first symptoms in the sweet lime, similar phenomena were observed in the sour orange seedlings, although here they were less severe. The damage was restricted to the pots with high concentrations of sulphate: 2293 and 1410 p.p.m., respectively.

The ultimate development of the plants is shown in Table II.

TABLE II

Development of the seedlings

Treatment No.	Sour orange				Sweet lime			
	I	II	III	IV	I	II	III	IV
SO_4^{--}, p.p.m.	2293	1410	540	381	2293	1410	540	381
Length increase (% of initial length)	166	177	178	174	125	133	149	137
Total fresh weight of plant (gm)	11.40	17.24	17.86	22.44	8.46	14.58	13.29	16.82
Fresh weight of roots (gm)	3.67	7.15	7.12	10.86	4.05	7.01	6.64	9.27
Fresh weight of leaves (gm)	5.75	7.56	7.59	8.51	2.86	4.86	4.26	4.67
Ratio tops/roots (fresh weight)	2.11	1.41	1.51	1.06	1.09	1.08	1.00	0.81

The table shows that a lowering of the *p*H to values near 4, which implied the addition of large quantities of sulphuric acid, actually exceeded the tolerance limit of citrus plants. On the other hand, the addition of sodium as carbonate, which was necessary to raise the *p*H to nearly 9, though at the beginning apparently not excessive, resulted

finally in taking up 22% of the total exchange capacity of the limited adsorbing complex of the sand.

In the course of the experiment the pots were watered to field capacity at appropriate intervals. The day after, the upper soil layer was stirred to improve aeration. The plants were measured several times and their condition was noted. The pots were arranged in randomized blocks and the results were subjected to a factorial analysis of variance.

At the end of the experiment, the plants with their roots were removed from the pots and the fresh as well as the dry weight of their organs was established. Shortage of material in part of the repetitions obliged us to pool the dried leaves of all repetitions of the same treatment in one sample for analysis. The results of the analyses therefore represent average figures, and the significance of differences between the treatments cannot be examined. Before drying, the leaves were carefully washed and ground into a fine powder in a Wiley mill. The results are averages of two analyses.

N was determined by the micro-Kjeldahl method. After wet incineration of the powder with concentrated nitric acid and 72% perchloric acid, the constituents of the ash were determined in the following ways: P by the colorimetric method with ammonium molybdate and ammonium vanadate, using the wave length of 4000 Å; S by precipitation with $BaCl_2$ and determination as sulphate; Ca, K and Na with a Beckman flame spectrophotometer; Mg colorimetrically by the titanium yellow method.

RESULTS

Effect of excess sulphate

The data in Table II demonstrate clearly the inverse relationship between the concentration of sulphates in the soil and the rate of growth of the plants.

The data further show a significant difference between the rate of growth of the sweet lime and the sour orange seedlings. This difference is apparent in all concentrations of sulphate, even the lowest. As will be shown later, this difference is due to the intolerance of the lime to sodium. Furthermore, the lime as a species of the lemon group is also more affected than the sour orange by the condition of high total salinity which obtained in the soil.

The growth of roots was gradually reduced by the excess of sulphate. In Table III we show this reduction in relation to the normal value (100) of seedlings grown with relatively low sulphate concentration. The figures show a greater relative reduction of growth of the roots than of the whole plants.

TABLE III

Relative rates of total growth and root growth under the influence of rising concentration of sulphate

Treatment		I	II	III	IV
Concentration of sulphate in the soil (p.p.m.)		2293	1410	540	381
Sour orange —	Total growth	46	76	79	100
	Root growth	34	65	65	100
Sweet lime —	Total growth	50	86	79	100
	Root growth	43	75	72	100

The change in the top/root ratio with rising concentration of sulphate is of considerable interest. This ratio becomes larger with the increase in intensity of poisoning of the plant; this phenomenon reflects the greater damage suffered by the roots which are in immediate contact with the poisonous agent and possibly also specifically less tolerant than the aerial organs.

TABLE IV

Concentration of chemical constituents in the leaves (% of dry weight)

	Sour orange					Sweet lime				
Treatment	I	II	III	IV	V	I	II	III	IV	V
SO_4^{--} (p.p.m.)	2293	1410	540	381	0	2293	1410	540	381	0
N	3.23	2.51	2.53	2.42	2.24	3.23	3.18	3.42	2.86	3.07
P	0.25	0.09	0.11	0.09	0.07	0.40	0.22	0.12	0.13	0.13
SO_3	1.61	1.18	1.00	0.92	0.89	1.23	0.98	0.81	0.87	0.61
K	1.31	0.95	0.90	0.87	1.37	1.09	0.90	0.84	0.80	1.06
Na	0.07	0.10	0.11	0.09	0.13	0.28	0.24	0.23	0.20	0.22
Ca	3.8	4.0	4.6	5.5	3.9	4.5	5.0	4.1	5.5	4.4
Mg	0.34	0.26	0.27	0.15	0.25	0.26	0.26	0.27	0.22	0.25

Interpreting the results of the chemical analysis, we must keep in mind that, together with the changes in concentration of salts in the soil, there were also changes in soil reaction which affected the absorption at the different concentrations. We wish, therefore, to insist mainly on those changes which must be considered as direct consequences of the concentration of sulphate. These changes are:

(*a*) *Accumulation of sulphate in the leaves.* Here, we find a pronounced rise of the concentration in the plant with rising concentration in the soil. The relationship is apparently of the logaritmic type, up to a point where the concentration of SO_3 in the leaf reaches about 1% of the dry substance and that in the soil — 1000 p.p.m. With a further rise in the latter, the concentration in the plants — sweet lime as well as sour orange — rises more sharply, which seems to indicate a flooding of the tissues beyond the control of the living protoplasm (See Figure 1).

This agrees with the findings of Chapman and Brown (1950), who consider a concentration of 1.0% sulphate in orange leaves as excessive.

The sour oranges accumulated more sulphate than the limes. According to data cited by Chapman and Kelley (1948), sour orange has been found to accumulate more sulphate than other citrus plants, also under ordinary conditions.

(*b*) *N, P and Ca content of the leaves.* The percentages of nitrogen and phosphate appear normal for citrus seedlings. There is a tendency to higher figures, especially of phosphorus, with rising concentration of sulphate; this points to synergistic rather than antagonistic relationship between phosphates and sulphates, in this case. In contrast to this, the absorption of calcium shows a definite inverse relationship to

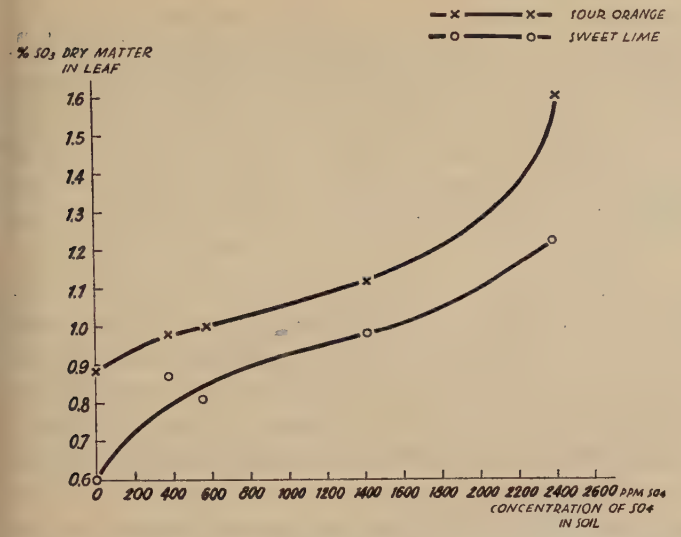


Figure 1

Effect of SO_4^{--} concentration on SO_3 concentration in leaves of sour orange and sweet lime.

that concentration. Analysing the relationship between Ca^{++} in the leaves and SO_4^{--} in the soil for both sour orange and sweet lime, it was found to correspond to the type of equation:

$$[Ca^{++}] = c\frac{1}{[SO_4^{--}]} + a$$

For sour orange, the equation was: $884\,(1/SO_4) + 3.26$. Its correlation coefficient $p = 0.95$ was significant at the 5% level. For sweet lime, no significance could be established (see Figure 2).

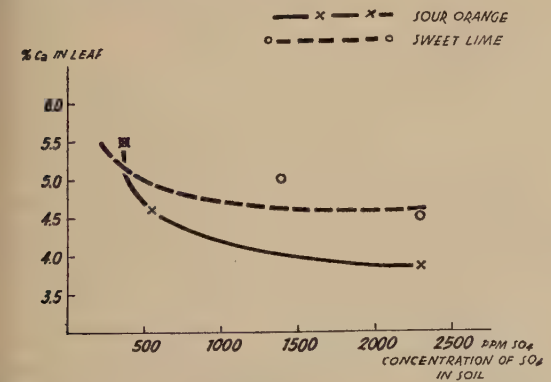


Figure 2

Effect of SO_4^{--} concentration in soil on Ca concentration in leaf (% of dry matter).

The results corroborate earlier findings of Eaton (1942) and Hayward et al. (1946). The possibility that the inverse relationship was a consequence of increasingly lower values of *p*H rather than a direct consequence of the rising SO_4^{--}, must be kept in mind.

Influences of excess sodium

(*a*) *Symptoms of toxicity.* About 50 days after the beginning of the experiments,

necrotic spots began to appear on the leaves of the sweet lime seedlings of all treatments. The spots developed mainly at the margins, but sometimes also at the tips. From a necrotic centre, the spots expanded concentrically towards the central vein, coalescing with each other and increasing in size, till the abscission of the leaf. As in the case of injury by sulphate, the mature rather than the younger leaves were affected and they were shed first (see Figure 4, right). The spots were sharply defined, without transitional zones to the neighbouring green tissue; the affected leaves did not become yellow.

Since the spots appeared at the same time and the same intensity in lime seedlings of all treatments, it seemed natural to assume that they were produced by excess sodium, since this element had been equally distributed in all the treatments, while total salinity varied and reached in certain treatments concentrations which are by no means excessive for citrus seedlings, judging from the conductivity of the concentrated extracts. Also Martin et al. (1953) report on "burns" on citrus leaves as a consequence of high concentrations of sodium in the absorptive complex, even if the concentration of electrolytes in the soil is rather low. The growing intensity of the phenomena during the experiment agreed well with the assumption that the causal agent was of a cumulative character, as would be the steady accumulation of sodium ions in the leaves.

(*b*) *Development.* From the data of Table II regarding the development of the seedlings, we learn that there was a significant difference between the respective development of the sweet lime and the sour orange, in favour of the latter, though Monselise (1953) has shown that, under normal conditions, the relative growth rate of sweet lime is superior to that of the sour orange. This demonstrates the greater sensitivity of the sweet lime to a high concentration of exchangeable sodium in the soil.

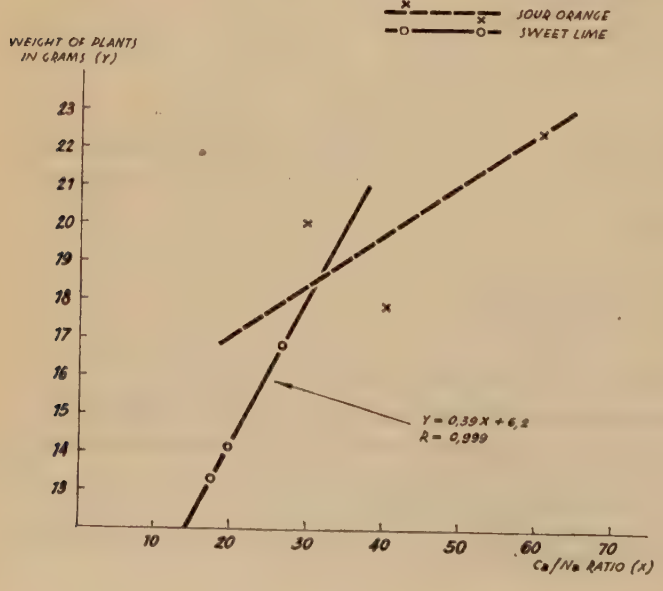


Figure 3

Relationship between the Ca/Na ratio in the leaf and the weight of plants at conclusion of experiment.

(*c*) *Concentration of sodium in the leaf.* The concentration of Na in the leaves of the sweet lime is greater than that in the sour orange. In the latter, the percentage figures fluctuate between 0.07 and 0.13% and may be considered normal, though rather high, while the values found in the sweet lime (0.20—0.26%) must be considered excessive.

Interesting results are obtained if we examine the ratio Ca/Na in the leaves and its relationship to the development of the plants. Figure 3 shows the results only for the range where the excess of sulphate does not play a decisive part. For both species the correlation between the above ratio and the development is positive, but it was found significant only in the case of the sweet lime. If we assume that in the investigated range the relationship is linear, as shown in the graph, then the steeper angle with the *X*-axis in the case of the sweet lime is a measure of the greater reactivity of this species to a lowering of the ratio Ca/Na, as compared with the sour orange.

DISCUSSION

(1) The assumption that the specific influence of the excess of sulphate on plants is confined to its inhibitory effect on the absorption of calcium, has been repeatedly discussed (1946). While in the present work rising concentrations of sulphate in the soil depressed the absorption of calcium decidedly, the figures for Ca in the dry substance of the leaf appear by no means abnormally low, but still fall in the range between 3.0 and 5.5% considered normal by Chapman and Brown (1950). Our lowest figure for calcium is 3.8%, while figures about 1.5% and lower would indicate a deficiency.

(2) On the other hand, the ratio Ca/Na in citrus leaves has been established to be highly important, and we can easily imagine that a reduction in the absorption of calcium — by high sulphate concentration or other influences — may prejudice the development of the plants by turning the balance in favour of the sodium. This will occur especially if the concentration of sodium in the plant is high.

(3) The citrus groves in Israel are irrigated during the growing season with large amounts of water and the quality of the irrigation water therefore has a decisive influence on the content of the exchangeable cations in the soil. For this reason, it seems to be highly important to determine the maximum SAR (sodium adsorption ratio) of the waters used for the irrigation of groves budded, as a rule, on sweet lime or on sour orange stock. If we accept the conclusion of Martin et al. (1953) that 14% exchangeable Na^+ is the maximum ESP (exchangeable sodium percentage) in the total exchange capacity, the highest permissible SAR will be in the neighbourhood of 5.5. (SAR is defined by the equation $SAR = Na^+/\sqrt{Ca^{++}+Mg^{++}/2}$).

The more the SAR of the irrigation water approaches this dangerous limit, the more will the use of sour orange stock instead of sweet lime be indicated. If the SAR is higher than 5.5, the water should be considered unsuitable for the irrigation of citrus groves. The critical figure depends, of course, also on the total salinity of the water. If this is high, the critical figure of SAR will become lower.

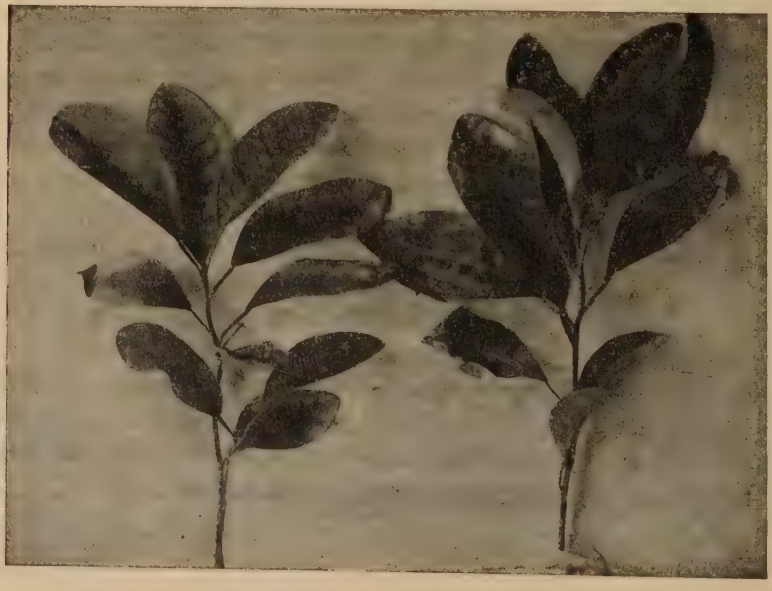
(4) In an article recently published by Aldrich et al. (1955) the authors report on an experiment undertaken in order to lower the *p*H of alkaline soil by sulphur, thus improving the conditions for the growth of lemons. This produced typical symptoms of injury by sulphate, although the authors endeavoured to remove the excess by ample irrigation. They arrive at the conclusion that application of sulphur for correction of alkaline soils is a dangerous undertaking. In the light of his own experience the present author is also inclined to think that the treatment with sulphuric acid of alkaline soils in citrus (and especially lemon or lime) groves endangers the trees by poisoning with sulphate ions.

REFERENCES

1. Aldrich, O. G., Buchanan, J. R. and Bradford, G. R., 1955, Effect of soil acidification on vegetative growth and leaf composition of lemon trees in pot culture, *Soil Sci.*, **79**, 427—441.
2. Bower, C. A. and Wadleigh, C. H., 1948, Growth and accumulation by four species of plants as influenced by various levels of exchangeable sodium, *Proc. Amer. Soc. Soil Sci.*, **13**, 218—223.
3. Chapman, H. D. and Brown, S. M., 1950, Analysis of orange leaves for diagnosing nutrient status with reference to potassium, *Hilgardia*, **19**, No. 17.
4. Eaton, F. M., 1942, Toxicity and accumulation of chloride and sulphate salts in plants, *J. agric. Res.*, **64**, 357—401.
5. Haas, A. R. C. and Thomas, E. E., 1928, Effect of sulphate on lemon leaves, *Bot. Gaz.*, **86**, 345—354.
6. Haas, A. R. C. and Brusca, J. N., 1954, Sodium effects in citrus seedlings, *Citrus Leaves*, **34** (8), 12—13.

6a. Hayward, H. E., Long, E. M. and Uhvits, R., 1946, The effect of chloride and sulphate salts on the growth and development of the Elberta peach on Shalil and Lovell rootstock, U.S.D.A. Tech. Bul. No. 922, 48 pp.

7. Martin, J. P., Harding, R. B. and Murphy, W. S., 1953, Effects of various soil exchangeable cations on growth and chemical composition of citrus plants, *Soil. Sci*, **46**, 285—295.
8. Monselise, S. P., 1953, Growth analysis of citrus seedlings, *Palest. J. Bot. Rehovot*, **8** (1), 54—75.
9. Reuther, W. and Smith, P. F., 1950, A preliminary report on the relation of nitrogen, potassium and magnesium fertilization to yield, leaf composition and incidence of lime deficiency in oranges, *Proc. Amer. Soc. hort. Sci.*, **56**, 27—33.
10. Salinity Laboratory Staff, 1945, Diagnosis and improvement of saline and alkali soils, U.S.D.A.
11. Webber, H. J. and Batchelor, L. D., 1948, *The Citrus Industry*, Part I.



Sweet lime seedlings injured by excess of sulphate (left) and excess of sodium (right). Sulphate produced interveinal chlorosis, sodium dry coalescing spots, originating at the margin.

PENETRATION ACTIVE DES RACINES DE BUISSONS MEDITERRANEENS DANS LES ROCHES CALCAIRES*

H. R. Oppenheimer

Faculté d'Agriculture de l'Université Hébraïque, Rehovot

ABSTRACT

Large mountainous stretches around the Mediterranean Sea have been laid bare by soil erosion. Pioneers of the vegetation settling on bare rock are not only lichens and mosses, and dwarf bushes belonging to the Labiatae, Compositae and other families, but also maquis shrubs and trees, as terebinths, lentisks, buckthorns and pines. Development of some species of the latter group is facilitated by their capacity to dissolve calcareous rock by acid excretions; this enables their roots to force their way into intact rock, instead of growing exclusively in fissures. Their tortuous passages resemble those of certain wood-destroying insects. It appears that roots of the lentisk play a preponderant part in this active disintegration of rocks which is of interest to foresters and fruit growers.

Il est bien connu que les pays du bassin méditerranéen ont perdu une grande partie du sol fertile qui couvrait autrefois leurs montagnes et collines; en conséquence, la végétation originelle a disparu de vastes étendues où la roche nue affleure à la surface. Ces maigres terrains ne se prêtent qu'au pâturage ou à l'afforestation. La forêt conserve ce qui reste de sol et contribue en même temps à la désintégration des roches grâce à l'influence des racines de ses arbres. Ces dernières se serrent dans les fentes des rochers qu'elles élargissent à mesure que leur diamètre s'accroit. Souvent on observe de gros blocs, pesant des centaines de kilogrammes, soulevés par les racines de chênes ou de pistachiers spontanés, ou bien on aperçoit dans des fissures les racines des pins d'Alep dont on connait les facultés d'adaptation aux terrains rocheux, secs et découverts. Même sur calcaire presque pur trouve-t'on le pin d'Alep, à condition que la roche ne soit pas trop dure.

Cependant on observe aussi la conquête des rochers par des végétaux spontanés de petite taille, arbrisseaux et arbustes nains vivaces. Leurs graines germent dans des cavités d'où leurs racines s'enfoncent et se ramifient à l'intérieur de la masse pierreuse. Incapables de faire éclater la roche, ces plantes remplissent de leur souches les cavités dans lesquelles ont germé les graines qui leur ont donné naissance et disséminent leurs semences qui engendrent d'autres individus. Ainsi se forment les associations rupicoles si caractéristiques des pays méditerranéens.

Nous sommes assez mal renseignés sur les qualités biologiques de ces espèces qui,

* Conférence lue au 8ème Congrès International de Botanique, Nice, 1954.

Received January 23, 1956.

Bull. Res. Counc. of Israel, Vol. 5D, 1956.

avec des lichens et des mousses, forment l'avant-garde de la végétation et la première des populations qui se succèdent pour aboutir soit au climax de maquis typique soit à un subclimax de garrigue. Cette lacune dans nos connaissances est regrettable parce que les végétaux en question sont des alliés de l'homme qui tendent à réparer les dommages causés par les dévastations et la négligence des siècles passés.

Dans la présente communication, nous allons relater quelques observations sur la flore rupicole de la région de Zikhron Jacob en Israël, région qui fait partie de la fameuse montagne du Carmel. Sur les pentes occidentales de cette montagne règne l'association à *Ceratonia siliqua* et *Pistacia lentiscus* définie par notre collègue, feu Alexandre Eig. Il s'y mêle des chênes (*Quercus calliprinos* Webb) et des térébinthes (*Pistacia palaestina* Boiss.), éléments dominants d'une autre association très caractéristique des sols de terra rossa et très répandue sur le territoire méditérraneen de la Palestine. Parmi les plantes enracinées dans les rochers, on trouve également *Rhamnus palaestina*. Sur des surfaces entièrement dénudées, on constate la présence du *Varthemietum iphionoides*, association litho- et héliophile où dominent des végétaux de petite taille appartenant au sous-domaine méditerranéen oriental. En dehors de *Varthemia*, Composée xérophile, très odorante et visqueuse qui fleurit à la fin de la longue saison sèche (fig. 1), citons *Micromeria juliana* et *serpyllifolia*, *Fumana arabica* et *thymifolia*, *Stachys palaestina* (fig. 3), également *Teucrium creticum* et *Hyparrhena hirta* moins caractéristiques de la roche nue. Les photographies ci-jointes vous aideront à concevoir de façon plus exacte leur aspect et leur développement en milieu rocheux.

On observe un phénomène très frappant et de grande importance dans le développement de cette végétation: l'expansion horizontale du lentisque à la surface des rochers. En effet, les branches de cet arbrisseau se dispersent dans toutes les directions à partir du centre enraciné formant finalement des coussins hauts d'un mètre et plus qui couvrent jusqu'à des centaines de mètres carrés (fig. 4). Le développement luxuriant de cette espèce connue pour son influence favorable sur la conservation du sol, démontre qu'il se plait dans la roche, comme du reste sur les falaises du canal de Corinthe et sur celles de la Côte d'Azur. Naturellement, on peut se demander si son système radiculaire a une extension semblable à celle de ses branches rampantes et s'il peut réellement être limité aux fissures de la roche ou s'il ne pénétrerait pas aussi la roche compacte. Autrefois, une telle hypothèse aurait été généralement écartée, car on était alors convaincu que les racines ne peuvent qu'élargir des fentes préexistantes, et qu'elles sont incapables d'attaquer la roche compacte. Cependant, nous avons pu décrire un cas de pénétration active des racines de pin d'Alep déjà en 1933 (Oppenheimer 1933). Il s'agissait alors de plants de pépinière non irrigués sur le Mont des Oliviers, plants dont les racines pénétraient à angle droit la craie sénonienne tendre. Plus récemment, Zohary et Orshansky (1951) ont trouvé des preuves de pénétration de racines de *Varthemia iphionoides*, *Stachys palaestina* et *Podonosma syriacum* dans la roche intacte. On se rend compte maintenant que ce phénomène qui parait être plutôt rare, dépend d'une part de la capacité que possèdent les racines de secréter des substances de réaction acide et, d'autre part, du degré de dureté et d'hétérogénéité des roches.

Sur les pentes de la montagne de Zikhron Jacob, on trouve des calcaires durs et des dolomies qui appartiennent aux formations cénomaniennes du Crétacé. Ces roches se trouvent dans tous les stades de décomposition et sont, de ce fait, plus ou moins friables et poreuses. Il y a aussi des conglomérats. Parfois la couche supérieure de ces roches apparaît formée par les résidus d'eau ayant évaporé à leur surface pendant la saison sèche. Ces résidus constituent une croûte appelée "nāri" en arabe.

L'échantillon de roche que je vous présente est probablement du nari. Vous pouvez aisément vous rendre compte qu'il est percé de forures dont le diamètre atteint plusieurs millimètres (fig. 5). La forme et les dimensions de ces forures rappellent celles que produisent les larves de certains lépidoptères xylophages, tels que *Zeuzera pyrina* ou *Cossus cossus* qui vivent dans les troncs d'arbres. Le parcours de ces galeries est très irrégulier, témoignant des obstacles que la racine tâtonnante a dû contourner pour progresser dans ce milieu hostile à sa croissance. Les végétaux auxquels ces racines appartiennent nous sont inconnus, et leur identification n'est pas chose facile même si l'on trouve, dans les forures, des racines plus ou moins bien conservées. Très souvent les racines s'effritent si l'on les coupe au rasoir, et leur identification d'après la structure forme le thème de recherches qui n'ont pas encore été achevées.

Dans les autres échantillons vous remarquerez des racines plus fines dans des forures plus étroites qu'elles ont produites en perçant la roche cristalline et blanche. Voici enfin des fragments de craie blanche présentant des taches grisâtres à la surface (fig. 6, à gauche). Il s'agit de fragments d'une roche qu'on a fait éclater à la dynamite. Caroubiers et lentisques poussent près de cette localité. En examinant à la loupe ces taches grises, on se convainct aisément qu'elles sont formées par les empreintes de très fins réseaux de racines qui ont disparu mais ont érodé de leurs secrétions le carbonate de la roche. Il semble probable que, au moins dans le cas où la roche environnante apparait toute blanche, ces racines n'ont pas crû dans des fissures préformées. Quoique nous n'en ayons pas la preuve formelle, il nous semble que les très fines racines qui croissent dans la roche comme si c'était de la terre meuble et compressible, sont celles du lentisque. Cette hypothèse qui est soutenue par l'aspect extérieur des racines et leur structure, nous expliquerait le rôle prépondérant que cette espèce omni-méditerranéenne joue dans la reconquête de terrains rocheux et leur couverture par la végétation. La photographie du système radiculaire d'un bel exemplaire de lentisque (fig. 9) que nous avons prise par dessous dans une grotte de calcaire très tendre près de la route de Zikhron Jacob à la station de chemin de fer de la même localité, donne une idée de son développement puissant et de sa ramification au fond. Cet arbrisseau avait la bonne fortune d'arriver à une couche amollie après avoir pénétré par un trou de la couche endurcie à la surface.

Parmi les espèces qui, à notre avis, vaudraient la peine d'être examinées du point de vue de leur capacité de croissance active à l'intérieur de roches, citons ici *Capparis spinosa*, le figuier et *Nicotiana glauca* que l'on trouve subspontané sur des roches en Grèce et en Palestine.

Schroeter (1926), qui s'est intéressé aux lithophytes des Alpes, a proposé le nom

de "rhizophagolithophytes" pour les plantes qui attaquent la roche intacte, par opposition aux "chasmolithophytes" dont les racines pénètrent dans des fissures dépourvues de terre. Tandis que sa classification nous semble satisfaisante, nous préféririons le terme de "lithophagophites" pour les premières, comme plus adéquat et plus simple. Le but de notre communicatlon était d'attirer l'attention des chercheurs du bassin méditerranéen sur ce phénomène.

En conclusion, qu'il nous soit permis de souligner que les capacités saxifrages et lithophages des plantes méditerranéennes offrent un intérêt pratique considérable pour le forestier et l'arboriculteur en général. Si nous savions utiliser ces plantes, et tout particulièrement les arbres et arbrisseaux, et favoriser leur développement par les mesures appropriées d'ensemencement et de dégagement, nous pourrions arriver à raccourcir considérablement le processus séculaire de transformation des roches en terre cultivable. Il nous serait alors possible de cultiver, comme stades secondaires de rotation, caroubiers, oliviers et amandiers, dans des lieux où actuellement une telle utilisation du terrain rocheux paraît impossible ou ne serait pas rentable.

BIBLIOGRAPHIE

1. Oppenheimer, H. R., 1933, *Gartenbauwiss.*, **7**, 308.
2. Schroeter, C., 1926, *Pflanzenleben der Alpen*, Zuerich.
3. Zohary, M. and Orshansky, G., 1951, *Palest. J. Bot. Jerusalem*, **5**, 119.

1

2

3

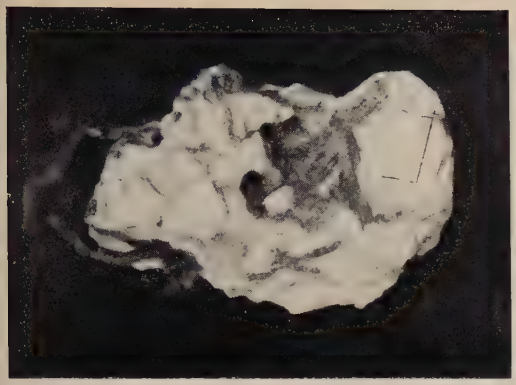
4

1. Végétation rupicole: Roche nue se couvrant de buissons de *Varthemia iphionoides* et *Pistacia lentiscus* (au fond).

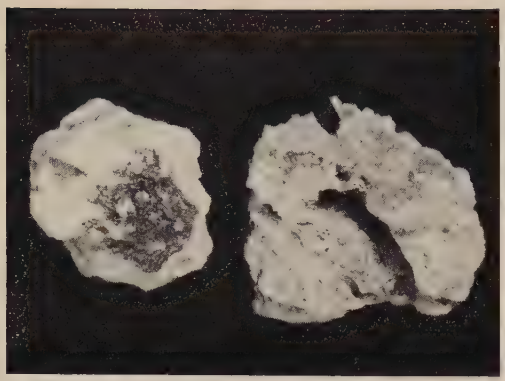
2. Végétation rupicole: *Micromeria serpyllifolia* à grandes tiges fleurissantes sortant d'un petit trou dans un bloc de calcaire dur.

3. Végétation rupicole: *Stachys palaestina* sur roche dur de dolomie.

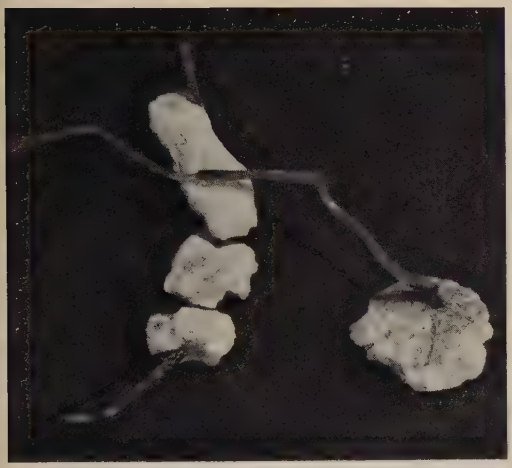
4. Coussins de lentisque couvrant la roche nue sur la montagne de Zikhron Jacob. Au fond la plaine et la côte de la mer.



5

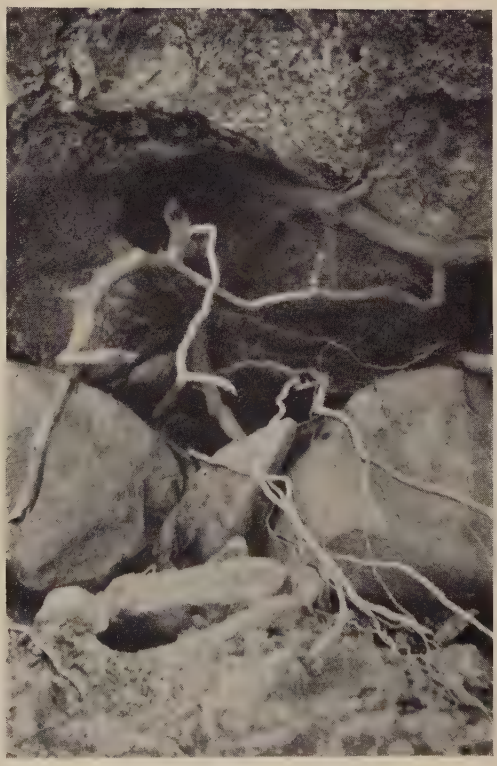


6



7

8



9

5. Racines épaisses (5 mm) de lentisque perforant un fragment de roche calcaire plutôt dur.

6. A gauche: Réseau de racines tres fines entamant la craie blanche par leurs excrétions acides. — A droite: Forures d'une racine épaisse et une autre plus mince (les deux probablement de lentisque) qui ont corrodé et perforé la roche poreuse. Au milieu on remarque l'écorce désintégrée de la première.

7. Racines minces (2 mm) de lentisque avec fragments de roche calcaire poreuse et fragile enfilées sur elles après éclatement de la roche entourante.

8. Racine de chene perforant roche calcaire et se ramifiant dans elle.

9. Racines de lentisque pénétrant par-dessus dans une cavité produite plus tard par influence humaine.

LETTER TO THE EDITOR

Influencia de hormonas vegetales sobre la germinación del polen de los citrus

Si bien mucho se ha estudiado la influencia de ciertas substancias sobre el poder germinativo del polen en distintas especies, especialmente en los frutales de hojas caducas, poco ha sido dicho al respecto en cuanto a los frutales cítricos se refiere. Los datos que aparecen a continuación han sido tomados de una tesis (M.Sc. Thesis) aún inconclusa, y los experimentos han sido llevados a cabo con polen de limonero de la variedad Meyers. Esta variedad es conocida como portadora de gran cantidad de polen, por cuya razón se lo considera un buen polinizador, teniendo además un período de floración larga y en cierta medida dispersa en las distintas epocas del año, además de la floración primaveral. Las experiencias de germinación en medios artificiales dieron resultados positivos en soluciones de sacarosa de distintas concentraciones. El método seguido es el de "hanging drop", que consiste en una gota de la solución colocada sobre el lado inferior de un cubreobjeto, y entre este y el porta-objeto un anillo de grosor variable, untado con vaselina en ambos bordes, formándose entre ambos un espacio cerrado que impide le evaporación del agua de la solución y el consiguiente aumento en su concentración. Los granos de polen son esparcidos sobre la superficie de la gota. Con el mismo principio se ha ensayado también con éxito la colocación de las gotas de la solución germinativa en platillos de Petri, del lado inferior de la tapa, colocándose en el interior del platillo discos de papel de filtro impregnados en agua, cuya finalidad es la de mantener elevado el grado de humedad ambiente. Este último método es mucho mas sencillo por cuanto el número de gotas puede ser mucho mayor. Para la posterior observación puede llevarse al microscopio tan sólo la tapa con las gotas, o las dos partes en conjunto, debiéndose en este caso concentrarse las gotas en el centro de la tapa, por sobre un corte practicado en el papel de filtro para permitir el pase de la luz.

Los árbolos elegidos para la experiencia formaban parte de la parcela experimental de limoneros de la Estación Agrícola Experimental de Rehovot, y en el momento de recolección de las flores se hallaban en buen estado y no presentaban síntomas de deficiencias nutritivas.

La recolección de las flores fué realizada en el mes de Enero de 1956. Las flores fueron recolectadas cuando comenzaban recién a abrirse, y las anteras se hallaban aún cerradas. Las anteras fueron separadas y colocadas en un recipiente de vidrio a baja humedad durante 48 horas, con lo cual se logró la completa apertura de las mismas y descubriéndose gran cantidad de polen, fácilmente separable de las anteras con un pequeño pincel.

La germinación fué realizada a 20°C en un termóstato, y la observación hecha al cabo de 24 horas.

Received April 25, 1956.

La determinación de los porcientos de germinación fué hecha como promedio de tres platillos de Petri con 5 o 4 gotas cada uno. Las experiencias demonstraron un porciente de germinación creciente con el aumento en la concentración de las soluciones hasta 25%, según puede verse en la tabla No. I.

TABLA NO. I

Concentración de la solución (%)	% de germinación			Promedio
	a	b	c	
5	7	6	7	6.6
15	25	16	22	21.0
25	26	31	26	27.5
40	27	30	25	27.2

En otra experiencia fueron agregadas la concentración standard (15%) distintas substancias y elementos hallados por distintos investigadores como activadores de la germinación en distintas especies.

En la tabla No. II pueden verse los resultados.

TABLA NO. II

Adición al medio germinativo	% de germinación			Promedio	Indice
	a	b	c		
——————— (Control)	16	22	25	21.0	100
2—4 D. 100 ppm	29	19	24	24.0	114.2
Tiamina 50 ppm	22	25	23	23.2	110.0
Tiamina 150 ppm	26	37	32	31.3	149.8
Ac. Borico 10 ppm	21	30	26	25.3	121.1
Ac. Borico 100 ppm	34	25	32	30.0	143.5
1) I.B.A. 50 ppm	28	33	34	31.5	150.7
I.B.A. 150 ppm	23	28	27	25.9	123.9

Los asteriscos indican un aumento significante revelado por el análisis estadistico.

(I) Acido Indol Butirico.

Según puede verse, existe una influencia positiva de ciertas substancias, cuya medida varía grandemente con concentración, y se requerirían nuevas experiencias para determinar cuál es la substancia y concentración capaces de aumentar a un máximo el porciento de la germinación.

M. E. RESNIK
División de Horticultura,
Facultad de Agronomía
de la Universidad Hebrea,
Rehovot

NOTICE TO CONTRIBUTORS

Contributors to the *Bulletin of the Research Council of Israel* should conform to the following recommendations of the editors of this journal in preparing manuscripts for the press.

Contributions must be original and should not have been published previously. When a paper has been accepted for publication, the author(s) may not publish it elsewhere unless permission is received from the Editor of this journal.

Papers may be submitted in English, French and Russian.

MANUSCRIPT

General

Papers should be written as concisely as possible. MSS should be typewritten on one side only and double-spaced, with side margins not less than 2.5 cm wide. Pages, including those containing illustrations, references or tables, should be numbered.

The Editor reserves the right to return a MS to the author for retyping or any alterations. Authors should retain copies of their MS.

Spelling

Spelling should be based on the Oxford Dictionary and should be consistent throughout the paper. Geographic and proper names in particular should be checked for approved forms of spelling or transliteration.

Indications

Italics

All symbols and text to be italicized should be underlined.

Capitals

Capital letters should be capitalized in the MS.

Stopping

Words to be stopped should be s p a c e d out in the MS.

Other specifications

Any other variations in type size or character should be indicated clearly in a legend preceding the MS.

Special care should be taken to record clearly relative height of symbols to the line. This is often more easily achieved in legible handwriting than typing. Indices and subscripts should be accurately placed. As far as possible formulae should be confined to one line, e.g. $\frac{1}{x}$ should rather be written $1/x$.

Greek letters should be indicated in a legend preceding the MS, as well as by a pencil note in the margin on first appearance in the text.

When there is any room for confusion of symbols, they should be carefully differentiated, e.g. the letter "l" and the figure "1"; "O" and "0".

Thermodynamic notation

The following notation should be used:

Internal energy	U	Work function	A
Enthalpy	H	Gibbs' function	G
Entropy	S	Chemical potential	μ

Mathematical punctuation

Decimal division is indicated by use of a full stop on the line, e.g., 1.000 (one, accurate to the third place). Division of thousands is made by use of a comma, e.g., 1,000 (one thousand). Multiplication is indicated by a full stop centrally placed, e.g. $8 \cdot 10^{12}$.

Abbreviations

Titles of journals should be abbreviated according to the *World List of Scientific Periodicals*.

Units are used in the abbreviated form, in the singular, and are not followed by a full stop (only in. is followed by a full stop). The following is a list of the more common symbols: mm cm m km cm^3 m^3 g mg kg sec min hr °K °C.

Summary

Every paper must be accompanied by a brief but comprehensive summary. Although the length of the summary is left to the discretion of the author, 3% of the total length of the paper is suggested.

References

In Sections A and C, and in Letters to the Editor in all Sections, references are to be cited in the text by number, e. g., ... Taylor[3] ..., and are to be arranged in the order of appearance.

In Sections B and D, the references are to be cited in the text by the author's name and date of publication in parenthesis, e.g., ... (Taylor 1932).... If the author's name is already mentioned in the text, then the year only appears in the parenthesis, e. g., ... found by Taylor (1932)... . The references in these Sections are to be arranged in alphabetical order.

The following form should be used ;

3. TAYLOR, G. I., 1932, *Proc. roy. Soc.*, A138, 41.

Book references should be prepared according to the following form:

4. JACKSON, F., 1930, *Thermodynamics*, 4th ed., Wiley, New York.

TYPOGRAPHY

In all matters of typography the form adopted in this issue should be followed. Particular attention should be given position (of symbols, headings, etc.) and type specification.

ILLUSTRATIONS

Illustrations should be sent in a state suitable for direct photographic reproduction. Line drawings should be drawn in large scale with India ink on white drawing paper, bristol board, tracing paper, blue linen, or blue-lined graph paper. If the lettering cannot be drawn neatly by the author, he should indicate it in pencil for the guidance of the draftsman. Possible photographic reduction should be carefully considered when lettering and in other details.

Half tone photographs should be on glossy contrast paper.

Illustrations should be mounted on separate sheets of paper on which the caption and figure number is typed. Each drawing and photograph should be identified on the back with the author's name and figure number.

The place in which the figure is to appear should be indicated in the margin of the MS.

PROOFS

Authors making revisions in proofs will be required to bear the costs thereof. Proofs should be returned to the Editor within 24 hours, otherwise no responsibility is assumed for the corrections of the author.

REPRINTS

Each author will receive 50 reprints free of charge, and additional reprints may be ordered at the time the first proof is returned. A table designating the cost of reprints may be obtained on request.

Orders in America should be addressed to Interscience Publishers Inc., New York, N. Y., and in England and Europe to Wm. Dawson & Sons, Ltd., Cannon House, Macklin Street, London, W. C. 2, directly or through booksellers.

Annual subscription per section (four issues): IL.4.000 ($5.50, £ 2)
Single copy IL.1.000 ($1.50, 12 s.)

BULLETIN OF THE RESEARCH COUNCIL OF ISRAEL
PUBLISHED BY THE WEIZMANN SCIENCE PRESS OF ISRAEL

PRINTED BY GOVERNMENT PRESS, JERUSALEM

SET ON MONOTYPE